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A NOTE ON PETROFF'S CULTURAL METHOD FOR THE ISOLATION OF TUBERCLE BACILLI FROM SPUTUM AND ITS APPLICATION TO THE EXAMINATION OF MILK.

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Examination of Sputum.

Previous to 1915 the chief means of isolating the tubercle bacillus from sputum was animal inoculation with subsequent cultivation of the recovered organism on some suitable medium, as, for example, blood serum and glycerol egg. Because of the time required, the uncertainty of animal inoculation, and the possibility of modifying the organism by animal passage, this method has proved impractical and unreliable.

Uhlenhuth and Xylander (1908) found that antiformin in certain concentrations is bactericidal for almost all bacteria but the bacillus of tuberculosis, and formulated their well known method for isolating tubercle bacilli in pure culture. Other investigators have adopted their suggestions in whole or in part, but with results as a rule far from satisfactory. Petroff's method was, therefore, indeed welcome. Since the appearance of Petroff's articles (1915) Williams and Burdick (1916) have described a similar method in which they employ a medium of their own. No reports on any duplication of their work have been seen by the writer, but in the few attempts that were made in this laboratory to repeat the work of Williams and Burdick no satisfactory conclusions could be arrived at.

The Petroff medium is prepared easily. It consists of whole egg 2 parts, fresh meat juice (beef or veal) 1 part, glycerol 5 per cent, and gentian violet 1:10,000. It is heated in the inspissator at 85°C. until coagulated, and for an hour at 75°C. on the 2 succeeding days.

The sputum to be examined is digested with an equal amount of 3 per cent sodium hydroxide at 37°C. from 20 to 30 minutes. The

mixture is then neutralized with normal hydrochloric acid and centrifuged. The sediment thus obtained is spread over the surface of the above medium.

In the 1st year that this method was employed Petroff isolated the tubercle bacillus 129 times from 135 specimens of sputum, or in 95.55 per cent of the cases, growth being obtained in 7 to 14 days after inoculation. In several instances he recovered the organism from sputum which had failed to reveal it by staining and direct microscopic examination. He examined feces also, but found it more difficult to isolate tubercle bacilli from this source, being successful only 19 times in the testing of 35 specimens (54 per cent). Although Keilty (1915, 1916) was not so successful as Petroff he expressed the belief that the method would prove itself to be of practical value. He obtained growth from 12 out of 18 samples of known tubercular sputa, only 4 being isolated in pure culture, however. Mitchell and Simmons (1915) examined 35 specimens from which tubercle bacilli were obtained pure in 80 per cent of the cases.

The present investigation is in part a duplication of the work of others on sputum examination. The method was the same as that described by Petroff, except for a few minor details. Some difficulties were at first encountered, such as contamination and drying of the medium, but these were soon largely overcome. All the specimens used were sputa which were procured through the Yale Medical School, and which were pronounced positive as the result of microscopic examination.

Thirty-seven specimens were examined, and from twenty-four, or 64.8 per cent, tubercle bacilli were isolated. The shortest time required for visible growth was 2 weeks, while the average period was from 3 to 4 weeks. In some instances colonies could not be detected in less than 6 weeks after the inoculation. It is apparent from these statements that much more time was required here to obtain the organism in culture than in the work of Petroff. These results, however, coupled with those of other experimenters, appeared so promising that it was deemed desirable to apply the method to the examination of milk, and perhaps later to milk products, meats, sewage, dust, and other agents or media which may be suspected of being infected with tubercle bacilli.

Examination of Milk.

With our growing belief in the intercommunicability of bovine and human tuberculosis, the problem of determining the presence of tubercle bacilli in milk by a quick and reliable method is one of great importance. The need of such a method is made all the more apparent by the work of Hess (1908) in New York, Anderson (1909) in Washington, and other investigators in widely separate localities, which showed that at least 6 to 10 per cent of the unpasteurized market milk in this country contains the organism of tuberculosis.

This is not only a public health problem, but also one of great significance from the broader economic standpoint. Tuberculosis often spreads very rapidly in a herd of cattle which then becomes a constant menace to other live stock receiving the infected milk, as well as to young children. According to statistics of the Bureau of Animal Industry about 10 per cent of the dairy cattle in this country are reactors to the tuberculin test, and 9 per cent of all the hogs that were slaughtered under Government inspection in 1916 were found to present evidence of tuberculosis (Mohler and Washburn, 1917). It has been estimated that the aggregate loss to the United States from tuberculosis among farm animals is $14,000,000 annually.

The only method of determining the presence of tubercle bacilli in milk has been that of animal inoculation. This has proved too expensive, slow, and unreliable, as there is always the possibility of losing the animal from acute non-tubercular infection. Staining and direct microscopic examination of milk or its sediment for tubercle bacilli can receive no serious consideration, on account of the relatively small number of bacilli present.

In the first endeavors to apply Petroff's method to the examination of milk, the milk was artificially infected with a stock culture of *Bacillus tuberculosis*. A small clump of the bacilli was finely mashed in 6 cc. of bouillon or salt solution; after prolonged shaking 4 cc. of this suspension were added to about 85 cc. of cream, from which 4 cc. quantities were then removed and placed in sterile centrifuge tubes. This material was digested with an equal volume of 3 per cent sodium hydroxide solution for 20 to 30 minutes at 37°C. After neutralizing and centrifuging, inoculations from both the fat and

sediment layers in the centrifuge tubes were made on the gentian-violet-egg-meat-juice medium of Petroff.

The first experiments were so successful that larger quantities of milk were inoculated with fewer organisms, and allowed to stand for 12 to 24 hours, some in the ice box and others in the incubator, before the samples were taken for testing. With these less abundantly inoculated samples positive results were obtained less frequently than in the preceding work.

Thirteen samples of milk were artificially infected with tubercle bacilli, four with the human and nine with the bovine type. All of the human and five of the bovine type were recovered, making a total of nine, or 69.2 per cent, positive isolations, a higher figure than that obtained for sputum. From each of these specimens either three or four portions of the milk were taken, making 51 tests in all, from which tubercle bacilli were recovered twenty times, or in 39.2 per cent of the portions tested.

It was impossible to obtain milk from cows having tubercular udders. Samples from only eight tuberculin-reacting animals were available; four of these had reacted 3 years previously, one being negative on second test. The cultural tests with these four samples gave negative results. The other four were from cows that had recently reacted; these likewise proved negative by the cultural method. Another sample of milk which was reported as coming from a cow having generalized tuberculosis was tested, but here again no positive results were obtained, either by the method of Petroff or by guinea pig inoculation.

Through the kindness of the bacteriologists of the New Haven Health Department, 59 samples of milk obtained from widely different sources and taken to the City Laboratory for routine testing were placed at the writer's disposal. Of this number five, or 8.4 per cent, yielded positive growths of tubercle bacilli. As a rule, when four samples were tested one was selected at random for guinea pig inoculation also. In this control experiment 2 cc. of the milk were injected, 1 cc. subcutaneously and the other intraperitoneally. Most of the animals died of acute non-tubercular infection, and of those which lived for several weeks only one showed signs of tubercular infection.

Twenty-nine samples of milk from the State Bacteriological Laboratory have been examined also, all giving negative results. If these samples are included with those obtained from the City Laboratory the percentage of positive cultures is lowered from 8.4 to 5.6.

The above results are fairly comparable with those obtained several years ago in this laboratory, when a bacteriological study of the market milk of New Haven was made, and 6 to 8 per cent of the milk was found to be infected with tubercle bacilli, according to the guinea pig inoculation method. Only six samples of milk purchased in the open market have been examined in the present investigation. The results were all negative.

The colonies of tubercle bacilli which appeared on the medium in the successful isolations from milk were usually small and few in number, hence they were often difficult to detect. However, the bacilli were always found in great numbers in these colonies, and presented the characteristic appearance when stained by the Ziehl-Neelsen method. In one of the successful isolations a guinea pig was inoculated with the acid-fast bacilli. It died 2 weeks after the inoculation, with a loss in weight of 28 per cent and presenting tubercular lesions of the mesentery and liver. The rapidity of action in the test animal confirmed the microscopic examination which showed the bacilli to be of bovine origin.

The morphological characteristics of all the organisms isolated from milk were such as to indicate that they were of the bovine type. The possibility of modifying the medium which has been so successfully used in the isolation of the human type to meet further the requirements of the tubercle bacilli of bovine origin was considered, therefore. In the work of Armand-Delille and his coworkers (1913) luxuriant growths of *Bacillus tuberculosis* were obtained in synthetic media containing certain amino-acids and diamines. It was thought desirable in this investigation to add various amino-acids, and also small amounts of sugar and phosphates to the gentian-violet-egg-meat-juice medium of Petroff. This study has not progressed very far, however, and various combinations of glycocoll, asparagin, glucose, and phosphates are still being tried out. Thus far it appears as if either glycocoll or asparagin favors the growth of the tubercle bacilli of the bovine type.

Certain obstacles must be recognized in attempts to apply the Petroff method to the detection and isolation of tubercle bacilli present in cow's milk, that are not encountered in the examination of sputum. In the first place, the experiments cannot be controlled satisfactorily, as there is as yet no reliable method of identification with which the new method may be compared. Furthermore, the relatively small numbers of tubercle bacilli which are probably present in samples of infected milk render their detection correspondingly difficult. And finally, the slowness and difficulty with which the bovine type of *Bacillus tuberculosis* is made to grow on artificial media are factors which must be reckoned with. However, further investigation of this problem should greatly reduce these obstacles.

SUMMARY AND CONCLUSIONS.

1. Tubercle bacilli were isolated by the Petroff method from 64.8 per cent of the sputa which were found to be positive by the direct microscopic method.

2. The organism was recovered from 69.2 per cent of the samples of milk that were artificially infected.

3. Of the milk obtained through the New Haven Health Department Laboratory 8.4 per cent of the samples were found to contain tubercle bacilli, while none of the twenty-nine samples from the State Laboratory gave positive cultures.

4. All the guinea pigs (thirteen), except one, which were used as control animals died without any visible signs of tuberculosis.

5. Petroff's method for the isolation of tubercle bacilli from sputum may be applied successfully to the examination of milk. With slight modifications, the method should prove constant and reliable. The need of such a method for the examination of milk and milk products is apparent.

I wish to express my indebtedness to the members of the City and State Bacteriological Laboratories for supplying samples of milk, to Dr. S. A. Petroff for his suggestions on technique, and finally to Dr. L. F. Rettger for his advice and criticism throughout the course of this investigation.

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Uhlenhuth and Xylander, Antiformin, ein bakterienauflösendes Disinfektionsmittel, *Berl. klin. Woch.*, 1908, xlv, 1346.

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THE BIOLOGICAL IDENTITY OF THE FRIEDLÄNDER BACILLUS.

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(Received for publication, June 18, 1917.)

Although immunological reactions have been applied to the identification of members of the *Bacillus mucosus capsulatus* group, the refractoriness of these organisms to agglutination by immune sera has stood in the way of a satisfactory classification on this basis.

In spite of the success of Landsteiner,[1] Babes,[2] Klemperer and Scheier,[3] and Bertarelli[4] in agglutinating Friedländer or rhinoscleroma bacilli with immune sera, other investigators (Clairmont,[5] Sicard,[6] Defalle,[7] and Porges[8]) have found these organisms inagglutinable in their native condition, so that recent workers have employed the method of Porges,[8] which is directed towards dissolving the mucoid capsule or hydrolyzing the protein material which holds the bacilli in an inagglutinable suspension. But this treatment by heating in acid solution has been found by Streit[9] to lead to an increase in the susceptibility of the bacterial

[1] Landsteiner, K., Ueber die Folgen der Einverleibung sterilisirter Bakterien culturen, *Wien. klin. Woch.*, 1897, x, 443.

[2] Babes, V., Ueber das Rhinosklerom, in Kolle, W., and von Wassermann, A., Handbuch der pathogenen Mikroorganismen, Jena, 1st edition, 1903, iii, 420.

[3] Klemperer, F., and Scheier, M., Ueber Identität der Ozaena- und der Rhinosklerombacillen mit Friedländer'schen Bacillen, *Z. klin. Med.*, 1902, xlv, 133.

[4] Bertarelli, E., Die Kapselbacillen, insbesondere ihre Systematik und die durch sie bedingten immunitären Reaktionen, *Centr. Bakteriol., 1te Abt., Ref.*, 1906, xxxvii, 343.

[5] Clairmont, P., Differentialdiagnostische Untersuchungen über Kapselbakterien, *Z. Hyg. u. Infectionskrankh.*, 1902, xxxix, 1.

[6] Sicard, A., *Compt. rend. Soc. biol.*, 1899, i, series 11, 813.

[7] Defalle, W., Sur le rôle de l'enveloppe des microbes, *Ann. Inst. Pasteur*, 1902, xvi, 602.

[8] Porges, O., Ueber die Agglutinabilität der Kapselbakterien, *Wien. klin. Woch.*, 1905, xviii, 691.

[9] S reit, H., Zur Frage der Agglutinierbarkeit von Kapselbacillen, *Centr. Bakte iol., 1te Abt., Orig.*, 1906, xl, 709.

suspension to normal sera and to spontaneous agglutination, as well as to immune sera. For this reason as well as for the variation in the length of treatment required to make different strains agglutinable, the method has not, in the hands of Streit[9] and Beham,[10] been found suitable for diagnostic purposes.

Cultures made to grow in a dry or capsule-free state, by development at low temperature (110°C.), or on potato agar, or by an artificial selection of the smaller and dryer colonies for several generations, have been found by Streit and Beham respectively to be agglutinated by immune sera. Since these dry cultures have been found by these writers, and by von Eisler and Porges,[11] to regain their capsulated mucoid state on animal passage, this simpler method does not appear suited to the recognition of bacilli in their most characteristic condition, as direct from the animal body.

EXPERIMENTAL.

The present study was undertaken as part of an immunological study of the capsulated Gram-negative bacilli. The material consisted of fifty strains, some isolated in this laboratory, and the rest collected from various sources.[12] All the organisms could be placed in the group comprising Friedländer's bacillus, *Bacillus mucosus capsulatus*, etc., and *Bacillus acidi lactici*, *Bacillus aerogenes*, etc.

For comparison of the cultural characteristics the organisms were grown in parallel streaks on dextrose agar in large (15 cm.) plates. In this way it has been possible to distinguish a Friedländer type, with colonies gray, translucent, of fluid, often syrupy consistency, and transparent watery margins, from an *aerogenes* type showing variations but having in common opaque ivory-white colonies, of a more pasty consistency. This cultural distinction is the main difference between the two organisms described by Kruse,[13] and may be

[10] Beham, L. M., Die agglutinatorischen Eigenschaften der Kapselbacillen und Anwendung der Serumagglutination bei den Trägern von Kapselbacillen, *Centr. Bakteriol., Ite Abt., Orig.*, 1912, lxvi, 110.

[11] von Eisler, M., and Porges, O., Ueber die Differenzierung der Kapselbakterien mit Hilfe agglutinierender und präcipitierender Immunsera, *Centr. Bakteriol., Ite Abt., Orig.*, 1906, xlii, 660.

[12] For these I am indebted to Dr. W. Rothberg of the American Museum of Natural History, Dr. J. G. Dwyer of the College of Physicians and Surgeons, Dr. E. G. Stillman of the Hospital of The Rockefeller Institute for Medical Research, and Miss M. Olmstead of the Presbyterian Hospital.

[13] Kruse, W., in Flügge, C., Die Microorganismen, Leipsic, 3rd edition, 1896, pt. 2, 185.

regarded as the basis of the classifications of Clairmont[5] and of Strong.[14]

Eleven of our fifty strains belong to the Friedländer type. The cultural difference in our experience has been constant both for young and old cultures, but can be recognized surely only when the different cultures are grown upon the same plate. The fluidity of the Friedländer strains varies somewhat upon different samples of media, and on blood agar the growth is more luxuriant and opaque, but the mucoid characteristic is retained in all cultures and we have not been able to observe distinct moist and dry growing phases. Two further strains show a translucent mucoid growth on dextrose agar, but resemble *Bacillus coli* on plain agar. One of these, No. 12, from the intestine forms gas from lactose; the other, No. 48, from a normal mouth forms acid but no gas on lactose and saccharose, and can be distinguished by this means also from the eleven type strains. It is possible that such variable strains as these have been observed by authors who note a drying out of the cultures on long continued cultivation or a relation between mucoid growth and the water and sugar content of the media (Fürst[15]). Other organisms of a more colon-like growth are distinguished culturally without difficulty.

The source of the Friedländer type strains, and their fermentation reactions with dextrose, lactose, and saccharose are given in Table I.

Rabbits have been immunized with killed suspensions of agar growths of Strains 3, 24, and 27. Intravenous inoculations were made every 4 or 5 days. Eleven of the fourteen animals died in the course of the treatment, all but two dying in from 2 to 6 hours after a dose slightly larger than the previous one. At autopsy the findings of distended lungs, petechial hemorrhages of the serous surfaces, and greatly lengthened coagulation time of the blood have pointed to an anaphylactic death.

In the blood of the seven animals examined it has been possible to demonstrate agglutinins for the homologous strain after 19 to 23 days of immunization. For testing agglutination 0.1 cc. of a broth

[14] Strong, L. W., Ueber die Kapselbacillen, *Centr. Bakteriol., Ite Abt.*, 1899, xxv, 49.

[15] Fürst, T., Untersuchungen über Kapsel- und Hüllenbildungen bei den sogenannten Kapselbakterien *Centr. Bakteriol., Ite Abt., Orig.*, 1910, lvi, 97.

TABLE I.

Series No.	Source.	Dextrose.	Lactose.	Saccharose.
3	Bronchopneumonia	Acid and gas.	No acid or gas.	Acid and gas.
4	Lung	" " "	" " " "	" " "
17	Ropy milk	" " "	" " " "	" " "
23	Ozena	" " "	" " " "	" " "
24	Normal mouth	" " "	" " " "	" " "
25	Lobar pneumonia, blood	" " "	" " " "	" " "
27	Meningitis	" " "	" " " "	" " "
36	Sputum; child with pneumonia	" " "	" " " "	" " "
37	Sputum; child with pneumonia	" " "	" " " "	" " "
47	Normal mouth	" " "	" " " "	" " "
50	" "	" " "	" " " "	" " "

culture has been used, in a total volume of 0.5 cc. of serum dilution. The titer has been low for most sera not exceeding 1:10, but for two of the three animals that survived a longer immunization values of 1:40 to 1:60 were found. The addition of the bacteria to concentrated serum, 1:1 or 1:5 dilution, produces an almost immediate coarse flocculation which soon settles and is compacted into a firm disc in the bottom of the tube. In higher dilutions the flocculation is finer, slower in appearing, does not form so firm a disc, and in the highest dilutions does not produce a clearing of the tube.

This result presents a striking similarity to the agglutination of *Pneumococcus mucosus* by the immune serum recently described by Wadsworth and Kirkbride.[16, 17]

Quantitative relations between serum and culture are strictly maintained so that 0.1 cc. of serum will cause complete agglutination of the same amount of culture whether it is in a volume of 0.2 or of 1.0 cc. The concentration affects only the speed of the reaction. The stability of Friedländer bacillus emulsions has been noted by Porges, Streit, and Bertarelli, and is so marked that clearing of broth or saline emulsions is impossible even with the highest centrifuge

[16] Wadsworth, A. B., and Kirkbride, M. B., A note on the production of antipneumococcus sera, *J. Exp. Med.*, 1917, xxv, 629.

[17] This immune serum was kindly placed at my disposal by the authors.

speed obtainable. This is due, as Porges[18] explains, to the presence of dissolved bacterial protein substance. The soluble material is present in broth cultures, which are found considerably less agglutinable than equally turbid suspensions of bacteria washed in a Berkefeld filter or by centrifugation. The following protocol illustrates this point:

Serum 24-A	1:10	1:20	1:40	1:60
Culture 24 (24 hr. broth culture)	±	—	—	—
Emulsion of washed bacteria from same culture	+++	+	±	—

The dissolved protein may be obtained by filtration from broth cultures as soon as turbidity has begun to appear, as early as 4 hours after planting. This substance reacts specifically with immune serum to give a heavy flocculent precipitate so that it is probable that it hinders the outflocking of the bacteria mainly by altering the quantitative relations between serum and substrate.

The viscid fluid obtained by washing out the peritoneal cavity of an infected animal is readily flocculated by immune serum, and capsules can be demonstrated in the agglutinated mass from this, as well as from broth cultures and washed bacterial emulsions. Examined in the hanging drop, the capsules appear in saline solution or normal serum only as halos of refraction, but on the addition of concentrated immune serum come immediately into prominence, swell to half the diameter of a red blood cell, and show a sharp non-refractile periphery. A similar observation was made by Landsteiner,[1] but appears to have been overlooked by later investigators. In weaker sera, agglutination may take place without the occurrence of this change. By the fuchsin-copper sulfate method the swollen capsules take a uniform heavy stain.

These observations do not support a morphological conception of the capsule as an impermeable envelope; they indicate rather that the

[18] Porges, O., Ueber die Beziehungen zwischen Bakterienagglutination und Ausflockungserscheinungen der Kolloide, *Centr. Bakteriol., 1te Abt., Orig.*, 1906, xl, 133.

peculiar properties of the Friedländer bacillus protein determine the nature and the extent of its reaction with immune serum.

The eleven strains belonging to the Friedländer cultural type were agglutinated by immune sera developed against strains from pneumonia, from meningitis, and from a normal mouth. For a given serum, the agglutination titer was approximately the same for all strains. There was no evidence on which to base a subgrouping; unfortunately the supply of high titer serum was exhausted before complete absorption experiments could be applied to this point. The remaining thirty-nine strains representing different cultural types were not agglutinated by any immune serum even in 1:1 dilution.

CONCLUSION.

We conclude therefore that this series of eleven lactose-negative organisms of the Friedländer type, grouped together by Perkins[19] on the basis of fermentation reactions represents a single biological group. It can be distinguished from *Bacillus aerogenes* and other similar bacilli by cultural, fermentative, and serological reactions. There appears to be a close analogy between this group and *Pneumococcus mucosus* in the possession of a fixed cultural type, and the behavior toward immune serum. Both represent apparently a single biological group. Unfortunately no immune sera have been developed against the two strains that grew in moist and dry phases; it is possible that with immune sera for these light could be thrown on the relation suggested by Fitzgerald[20] that the capsulated bacilli represent a parasitic development of the *Bacillus coli* group.

I wish to express my indebtedness to Professor Zinsser for his suggestions, and my appreciation of the interest and advice of Professor J. Gardner Hopkins, both of the Department of Bacteriology of the College of Physicians and Surgeons of Columbia University.

[19] Perkins, R. G., *J. Infect. Dis.*, 1904, i, 241.
[20] Fitzgerald, J. G., *J. Infect. Dis.*, 1914, xv, 268.

THE EFFECT OF ALCOHOL ON THE REPRODUCTIVE TISSUES.

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Plates 57 to 60.

(Received for publication, July 24, 1917.)

That alcohol may produce distinct anatomical changes in the testicles of those who use it in excess has been observed from time to time by pathologists for many years, and yet this fact does not seem to be widely known or generally taken into account in considering the effects of alcoholism.

HISTORICAL.

As long ago as 1837, Rösch[1] described degenerative changes in the testicles of alcoholics, and Lancereaux described parenchymatous degeneration of the seminal tubules. In 1877 Schlemmer[2] discussed the effects of alcoholism on the testicles, and noted that it often caused fatty degeneration of the seminal epithelium, decrease in the number of spermatozoa, and pigmentation of the seminal fluid. Rheinstaedter[3] (1879) and Curschmann[4] attributed loss or decrease in testicle function to chronic alcoholism, the former stating that in his experience excessive beer drinking had a greater effect than wines or distilled liquors.

Busch[5] (1882) studied the testicles in 100 consecutive autopsies of adult males and found 27 without spermatozoa, 39 with few, and 34 with many. Although phthisis and other chronic diseases furnished many cases of azoospermia, he observed that this condition occurred frequently in alcoholics, especially when associated with cirrhosis.

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[1] Rösch, cited by Forel.[13]

[2] Schlemmer, A., *Vrtljschr. gerichtl. Med.*, 1877, xxvii, 444.

[3] Rheinstaedter, cited by Busch.[5]

[4] Curschmann, cited by Busch.[5]

[5] Busch, A., *Z. Biol.*, 1882, xviii, 496.

In 1898 Simmonds[6] reported the study of 1,000 male bodies, of which 125 showed azoospermia. As previously observed by Busch, Simmonds found chronic diseases responsible for many cases, the incidence of azoospermia in cancer cases being 7 per cent, in chronic nervous diseases 14 per cent, in phthisis 18 per cent, while of the chronic alcoholics 60 per cent had no spermatozoa. Of all autopsied males, 5 per cent were sterile through the use of alcohol, as against 3.3 per cent from genital diseases. He also found that mechanical occlusion of the seminal tract, even of years' duration, seldom causes atrophy of the testicles or absence of spermatozoa in the tubules.

Bertholet[7] (1909) made a report of the findings in 75 bodies of men dying at from 15 to 91 years of age, of whom 39 were habitual drinkers. Of the 39 alcoholics, only 2 had testicles that did not show atrophy of the parenchyma and sclerosis of the stroma. The most marked change occurred in alcoholics who died of tuberculosis or cirrhosis. Of the two alcoholics without atrophy, one was a man but 24 years old, and the other died of an acute intestinal obstruction. Bertholet describes the changes in detail. Generally the testicles are noticeably small and firm, because of atrophy of the epithelium and increase in the fibrous tissue. The spermatogonia are highly atrophic, the nuclei cannot be stained distinctly with hemalum, and it is impossible to distinguish between spermatocytes and spermatids, because they are all in the resting stage and show no mitosis or spermatozoa production. The cells of Leydig, on the other hand, seem to be increased in size, number, and staining intensity of the cytoplasm. The basal membrane is much thickened by concentrically arranged lamellæ and spindle cells; there is also an increase in the connective tissue between the tubules, with sometimes a slight round cell infiltration. The atrophy and azoospermia was complete in 24 of the 37, including 7 who died of cirrhosis; in but 4 was the change of only insignificant degree. Although similar changes of slight degree were found in non-alcoholics, the advanced changes were seen only in those who drank heavily. Even in senility the atrophy and fibrosis were less marked than in the alcoholics.

Since the reports of Simmonds and Bertholet, a few other papers have appeared corroborating their observations in all essentials, although not all investigators on alcoholism have considered the sex cells. Thus Fahr,[8] in his study of the anatomical changes in 309 alcoholics, makes no mention of the generative tract or the occurrence of changes in these tissues in the guinea pigs that he subjected to experimental alcoholism.

Weichselbaum and Kyrle[9] (1912) have furnished one of the best contributions to this subject, having carefully studied the testicles of 67 habitual alcoholics. They entirely support Simmonds' and Bertholet's conclusion that chronic

[6] Simmonds, *Berl. klin. Woch.*, 1898, xxxv, 806.

[7] Bertholet, E., *Centr. allg. Path. u. path. Anat.*, 1909, xx, 1062.

[8] Fahr, *Verhandl. deutsch. path. Ges.*, 1909, xiii, 162.

[9] Weichselbaum, A., and Kyrle, J., *Sitzungsb.-Ber. Akad. Wien.*, 1912, cxxi, 51.

alcoholism constantly causes retrogressive changes in the testicles independent of senility or chronic diseases, even to the point of complete loss of the seminal epithelium. Also they corroborate the observation that the most marked changes occur in the testicles in alcoholism with cirrhosis. They too find that tuberculosis and cancer may produce similar changes, usually of mild degree, but alcoholism of itself produces marked changes without the coexistence of any other disease. Their paper contains a detailed description of the histological changes observed.

Because of the repeated observation that alcoholics with cirrhosis show especially marked changes in the testicles, Schopper[10] investigated the effect of liver injury on the testicles. He found that animals (rabbits, dogs, and rats) from which a large portion of the liver had been removed, or in which the liver had been injured by exposure to Roentgen rays, exhibited marked changes in the testicles. These changes consisted in a cessation of spermatogenesis, transudation into the interstitial tissue, desquamation of the epithelial cells with formation of multinucleated cells, and even complete loss of the epithelium with increase in the interstitial cells.

In connection with his studies on the effect of parental alcoholism on the development of the offspring, Stockard[11] stated that guinea pigs given alcohol for as long as 19 months by inhalation, and therefore in unknown quantity, were still good breeders, and when killed showed no changes in the testicles. Ovaries of females similarly treated also showed no recognizable structural changes. On the other hand, Riddle and Basset[12] found that doves and pigeons made to inhale alcohol 1 to 2 hours daily for a few weeks produced eggs with yolks smaller than those in the eggs laid before the alcoholism. In general, however, the possible anatomical changes in the ovaries seem not to have been considered by pathologists, and the only mention we can find of this topic is contained in the review of Forel,[13] who says, "more recently Bertholet has also demonstrated atrophy of the ovaries and ova of an alcoholic woman," but no reference is given and we have been unable to locate this statement in Bertholet's writings.

The only investigation of the influence of experimental alcoholism on the testicles of animals that we can find, beyond Stockard's negative results, is described by Bouin and Garnier,[14] and unfortunately this report concerns only an isolated observation. It is stated by these authors that in the course of experiments on the effect of chronic alcoholism on the white rat, marked alterations were observed in the testicles of two animals that had been fed alcohol for 8 and 11 months respectively. No mention is made of how many animals were studied in

[10] Schopper, K. J., *Frankfürter Z. Path.*, 1911, viii, 169.

[11] Stockard, C. R., *Arch. Int. Med.*, 1912, x, 374; *Arch. Entwcklngsmechn. Organ.*, 1912–13, xxxv, 3. Stockard, C. R., and Papanicolaou, G., *Am. Naturalist*, 1916, i, 65.

[12] Riddle, O., and Basset, G. C., *Am. J. Physiol.*, 1916, xli, 425.

[13] Forel, A., *Münch. med. Woch.*, 1911, lviii, 2596.

[14] Bouin, P., and Garnier, C., *Compt. rend. Soc. biol.*, 1900, lii, 23.

all, or whether the other animals showed any changes in the testicles. In these two animals there was marked atrophy of the seminal tubules, often with almost complete disappearance of the germinal epithelium, and in none was the epithelium normal. Between the tubules was found an accumulation of lymph, but no fibrosis or arteriosclerosis. In the lumen of the tubules few spermatozoa were found, but there was much desquamation of the epithelium, and often fusion of the cells into masses. Pyknosis and karyolysis were common, and the Sertoli cells often showed amitotic division. Before degenerating completely the germinal cells pass through various stages of altered vitality, which, the authors suggest, explains the frequency of abnormalities in the offspring of alcoholics. We cannot find that these authors have published elsewhere further details of their investigations in this field.

General Physical Changes Due to Alcohol.

In a series of rats fed alcohol for varying periods of time to determine the effect of alcohol on the psychology of their offspring, an opportunity was given to observe the effect of chronic alcoholism on these animals.[15] Altogether we examined 15 male rats thus treated, 6 male rats of the second generation derived from them, 18 male rats of the third generation, and 8 male rats of the fourth generation. Approximately the same number of females was also studied. From each animal, with a few exceptions, the following organs were studied: lung, liver, kidney, spleen, stomach, heart, and sex glands. The animals were all killed, while in apparent health, with illuminating gas. They were all of about the same age, 6 to 9 months, except in the case of the rats fed 0.5 cc. for 10 months; these rats were 13 months old. All had been fed and cared for under identical conditions except for the administration of alcohol admixed with the food in quantities of from 0.25 cc. to 2.25 cc. per day, for periods varying from 2 to 10 months. The effect of the alcohol on the rats receiving it was, except for those fed for very long periods, to lessen learning capacity in almost direct proportion to the amount of the dose and the duration of the period during which it was administered.

The effect on body weight was also marked. Animals receiving 2.25 cc. *per diem* rate either gained weight far more slowly than the normal animals, or, as in some instances, actually lost weight though in the

[15] The results of the psychological study will be published shortly in *Psychol. Monog. Suppl.*

growing period. As for the offspring, the observed physical effects were, in the case of the offspring of rats receiving 0.5 cc., a decrease in size and lessened fertility. This was accompanied by a marked decrease in learning capacity. The offspring of animals receiving 0.25 cc. showed no marked physical changes and were, as to intelligent behavior, generally on a par with, if not actually superior to, the offspring of normal animals.

The microscopic changes in all structures except the sex glands may be summarized briefly. Nearly all showed more or less pulmonary infection of the type common in rats, consisting in the less advanced stages of a chronic bronchitis with much cellular infiltration and hyperplasia of the bronchial walls. Many showed extension of the process to the adjacent alveoli with a frank mucopurulent bronchopneumonia. The degree of this change seemed to have little effect on the conditions in the other organs, and bore no relation whatever to the changes observed in the sex glands.

In the liver there were often varying degrees of hydropic vacuolization of cells, this condition occurring in about half the specimens. Focal necrosis was found in about one-fourth, severe in but a few. These changes bore no reference to the severity of the lung infection or to whether alcohol had been administered, as shown by tabulation and comparison of the results. Occasionally a slight periportal round cell infiltration was found, but this also was independent of other conditions. Even in the rats that received large quantities of alcohol for some months, the livers showed no more change than in the others. There was nothing found at all resembling cirrhosis.

The kidneys showed commonly a slight degree of vacuolization of the tubular epithelium, which was possibly slightly more general in the alcohol animals than in the others. Two animals in which the kidneys showed slight formation of granular casts had received much alcohol. That the alcohol was the cause of the renal changes is probable, especially in view of the great amount of cast formation commonly observed in the urine of patients with acute alcoholism, but this is by no means certain.

In the stomachs of a few of the rats receiving alcohol there seemed to be slightly more round cells in the mucosa and submucosa than normally, but the changes were not marked enough to be considered as

distinctly inflammatory. In two of the alcoholized rats the gastric glands showed more mucus content than was seen in any of the other animals, and hence this change may be ascribable to the alcohol. The slight effect of the alcohol on the stomach of the rats corresponds to the relatively normal condition of the gastric mucosa of human alcoholics observed by Hirsch.[16]

The heart, spleen, and adrenals usually showed no changes, and in no instance changes that occurred in the alcoholized rats and not in the others.

Pathological Changes in the Reproductive Tissues.

Only the testicles showed definite changes, but here the effects were so marked, and so nearly constant, that they stand out conspicuously as the result of the alcohol feeding. Of the fifteen male rats treated with alcohol, the testicles of not more than two or three failed to show noteworthy changes, and only one could be called normal. Several different types of changes were observed, and one that attracted attention at once was an apparent decrease in the size of the seminiferous tubules, and in several instances a distinct decrease in the size of the testicle itself. Measurements were made of the tubules in the testicles from thirty-seven rats, twenty-five tubules being measured from each testicle to secure adequate representation, and then the average diameter was calculated.[17] The results of measurements in the several groups are given in Table I.

TABLE I.

No. of animals.	Minimum diameter.	Maximum diameter.	Average diameter.
6, 2nd generation rats	16.00*	19.16	17.26
18, 3rd " "	15.08†	22.60	17.50
8, 4th " "	15.56	18.32	17.08
15, alcoholized rats	10.00	18.24	14.65

* The measurements as recorded are the number of ocular micrometer spaces, not translated into microns. Each ocular micrometer space = 0.0155 mm.

† One very abnormal testicle in this series, measuring but 12.12, is not included.

[16] Hirsch, E. F., *Arch. Int. Med.*, 1916, xvii, 354.

[17] For these measurements we are indebted to Miss C. L. Chapin.

These measurements show at once that there is a distinct decrease in the size of the seminal tubules of the rats that had received alcohol, although in a few rats this change is not present. However, as Table II shows, but two of the fifteen rats that received alcohol had as large tubules as the average seminal tubules of their descendants that had not received alcohol. It also shows that the decrease in size does not vary directly with the amount of alcohol the rat had received. The tubules of normal rats of other strains corresponded in size to the tubules of the second, third, and fourth generation rats.

TABLE II.

Rat No.	Daily dose.	Time.	Diameter of tubules.	Histological changes summarized.
	cc.	*mos*		
1	0.25	2	13.40	Many defective spermatozoa; number decreased.
2	0.25	2	15.04	Spermatozoa decreased.
3	0.25	4	13.68	Few spermatozoa; mostly defective.
4	0.5	3	17.12	Nearly normal.
5	0.5	3	16.16	A few inactive tubules; mostly normal.
6	0.5	3	18.24	" " " " " "
7	0.5	10	11.32	Marked atrophy with intertubular edema; almost no spermatozoa, and these defective.
8	0.5	10	16.76	Much intertubular edema; most of tubules nearly normal, but number of spermatozoa decreased.
9	2.0	6	14.32	Very few spermatozoa; much desquamation.
10	2.0	6	14.16	Much desquamation and degeneration of epithelium; small number of spermatozoa.
11	2.25	3	10.00	No spermatozoa; great desquamation and degeneration of epithelium.
12	2.25	3	13.56	Only one-third to one-half the tubules contain spermatozoa, and few of these are normal.
13	2.25	6	15.84	Some edema; spermatogenesis much reduced.
14	2.25	6	13.88	Much atrophy and edema; spermatogenesis about 50 per cent of normal.
15	2.25	6	16.24	About three-fourths of the tubules nearly normal.

From Table II it will be seen that the least changes were found in the three rats (Nos. 4, 5, and 6) that received 0.5 cc. of alcohol for 3 months, while much more marked changes occurred in the three animals that received but 0.25 cc. of alcohol. Also, three of the rats that received the relatively enormous dosage of 2.25 cc. per day for

6 months, which would be equivalent to a man's taking over 2 quarts of whisky daily for a large part of his life, showed less change in the testicles than some of the rats that received somewhat less alcohol.

The changes produced by alcohol seem to take place in definite order. At first the spermatocytes seem to be normal in number and appearance, but there is soon an increase in the number of spermatids in the tubules with a decrease in the number of spermatozoa (see Fig. 1, showing a normal testicle, and Figs. 2 and 3, showing the testicles of rats which received 2 cc. and 2.5 cc. respectively). At the same time, or earlier, there is observed a greater diminution in the number of complete spermatozoa with tails than in the number of sperm heads. Not all the tubules in the testicles show the same degree of change, except in the most advanced cases (Fig. 4). In the more moderately affected testicles one finds some tubules nearly or quite normal, while others show merely a few active spermatocytes with the lumen packed with spermatids without spermatozoa or even spermatozoa heads (Fig. 5). Apparently the first effect of the alcohol is to render the formation of spermatozoa incomplete, so that the heads are formed without normal tails. In accordance with this, in one of these testicles we usually find the number of spermatozoa in the tubules of the epididymis much less than normal, and less in proportion than the number of spermatozoa seen in the seminiferous tubules, suggesting that the latter are too imperfect to migrate to the epididymis. The next effect of the alcohol seems to be to prevent the transformation of the spermatids into spermatozoa, whereby the tubules become filled with accumulated spermatids, with but a few spermatozoa or none at all. These spermatids then undergo a process of degeneration, so that they lose their nuclear stain and become granular. In the most advanced stages the tubules contain but the marginal cells, with few or no spermatocytes or spermatids (Fig. 6). Occasionally in the lumen of tubules showing advanced degeneration are found large cells with many nuclei or chromatin masses.

The degree of atrophy does not exactly parallel the decrease in functional activity, but usually the diameter of the tubules corresponds somewhat with the loss of function. When the tubules are atrophied there is commonly some compensatory accumulation of

serous fluid in the interlobular spaces, an edema *ex vacuo* (Fig. 4). In no instance have inflammatory changes been seen, and no marked fibrosis, although in some instances there has been an apparent slight increase in the thickness of the basement membrane of the tubules. We have noted no definite multiplication or other changes in the interstitial cells of Leydig, although when the tubules shrink apart these cells become more evident. No arterial changes were observed.

The epididymis generally reflects, in the number of spermatozoa it contains, the degree of functional impairment of the testicle (Figs. 7 and 8). In two of the rats there was also noteworthy desquamation of the epithelial lining of the ducts. Other changes were not observed in the epididymis.

Although the ovaries of the alcoholized females, and of the offspring of alcoholized parents, were examined in considerable numbers, it is not possible to make any positive statement as to the effect of alcohol on this organ. The changes of ovulation, pregnancy, and corpus luteum formation produce such great structural alterations in the ovary that we were unable to determine satisfactorily what changes were caused by the alcohol. There were two distinctly atrophic ovaries found in females that had had alcohol, and in several alcoholic females the ovaries were poor in ova in the sections examined.

Without study of serial sections of every ovary no conclusions of value could be reached from the material, and we were unable to undertake this task.

SUMMARY.

Administration of alcohol in the food of male white rats for 2 or more months, in daily quantities of 0.25 to 2.25 cc., results almost constantly in the appearance of marked degenerative alterations in the testicles. These changes affect the steps of spermatogenesis in inverse order to their occurrence, so that for some time before sterility and complete aspermia result, the animal is producing spermatozoa with all possible degrees of abnormality and deficiency. The possible relation of this abnormal spermatogenesis to the production of defective offspring is obvious. Individual rats show marked differences in the degree of change produced by equal amounts of alcohol. The

fibrous, interstitial, and vascular elements of the testicle are not affected, except for intertubular edema compensating for tubular atrophy. These experimental observations harmonize with the necropsy findings in human alcoholics. No other tissue was found to be noticeably affected by the alcohol; especially to be remarked is the absence of cirrhosis or fatty infiltration in the liver.

EXPLANATION OF PLATES.

PLATE 57.

FIG. 1. Normal testicle of a rat. × 58.

FIG. 2. Testicle of a rat which had received 2 cc. of alcohol daily for 6 months. Marked loss of germinative epithelium and absence of spermatogenesis are shown, without much decrease in size. Many tubules in this testicle also show spermatogenesis. × 58.

PLATE 58.

FIG. 3. Testicle of a rat which had received 2.25 cc. of alcohol daily for 3 months. Marked atrophy of the cellular content, decrease in size of the tubules as a whole, and complete absence of spermatogenesis are shown. × 58.

See higher power of the same in Fig. 6.

FIG. 4. Testicle of a rat which had received 0.5 cc. of alcohol daily for 10 months. Marked decrease in size of the tubules with intertubular edema. × 58.

PLATE 59.

FIG. 5. Tubule of a rat which had received 0.25 cc. of alcohol daily, showing absence of spermatozoa and accumulation of spermatids. This is one of the early stages of the degeneration of the testicular tubules. × 309.

FIG. 6. Higher power of Fig. 3. Later stage of degeneration. The tubules contain no spermatozoa or spermatids, and almost no spermatocytes. × 309.

PLATE 60.

FIG. 7. Normal epididymis of a rat. Tubules filled with spermatozoa. × 58.

FIG. 8. Epididymis of a rat which had received 2 cc. of alcohol for 6 months, showing great atrophy and no normal spermatozoa. × 58.

FIG. 1.

FIG. 2.

(Arlitt and Wells: Effect of alcohol on reproductive tissues.)

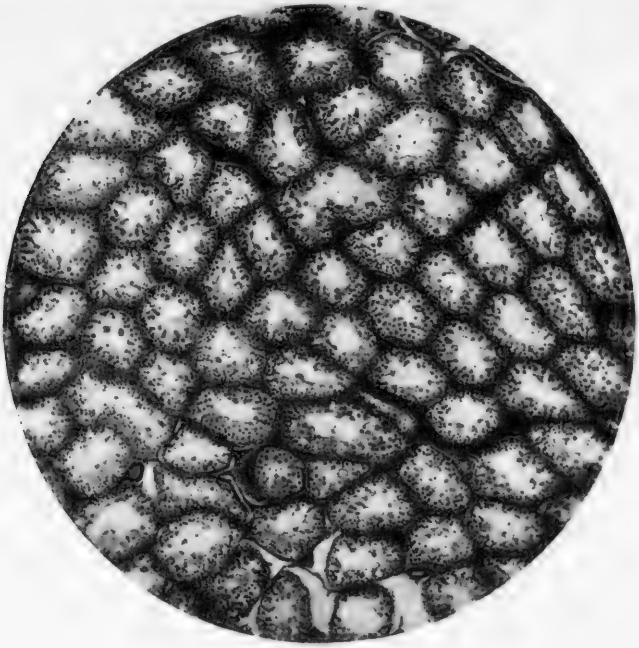

FIG. 3.

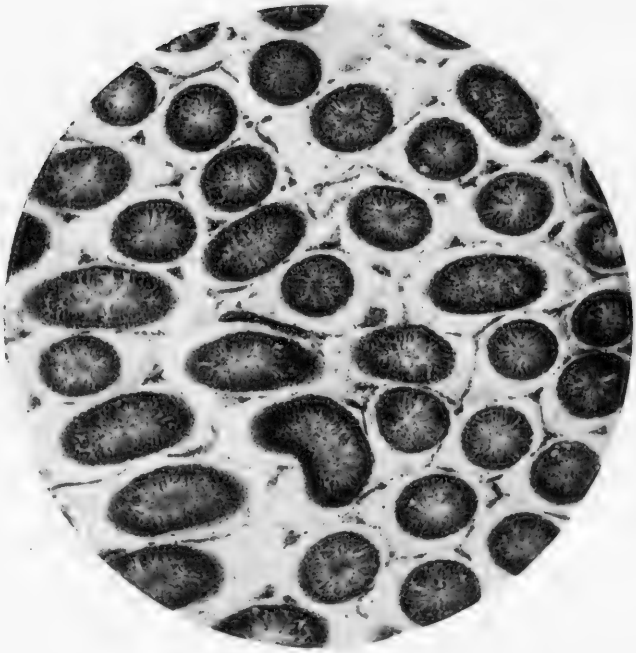

FIG. 4.

(Arlitt and Wells: Effect of alcohol on reproductive tissues.)

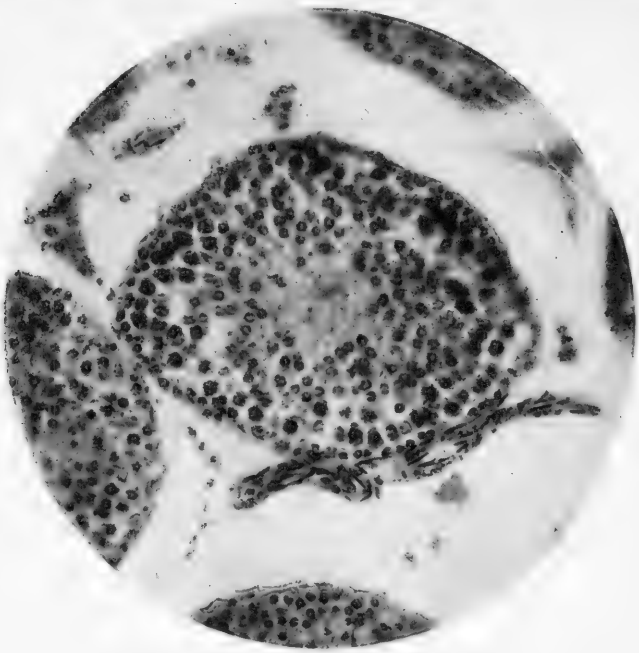

FIG. 5.

FIG. 6.

(Arlitt and Wells: Effect of alcohol on reproductive tissues.)

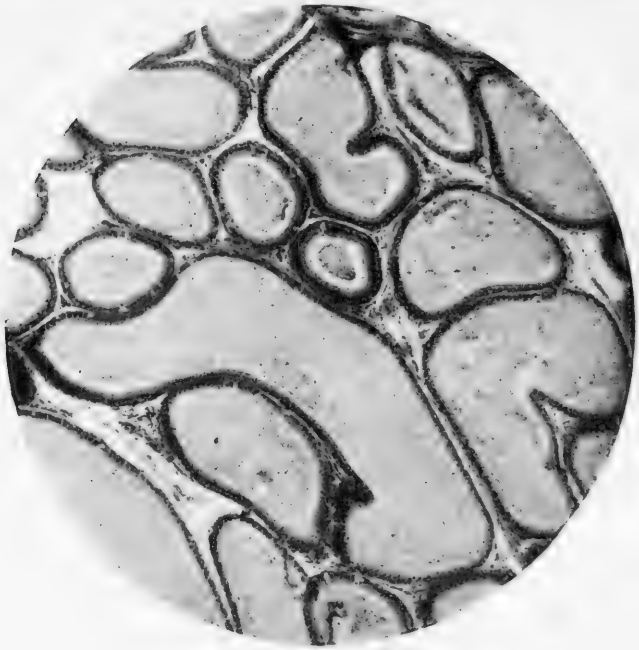

FIG. 7.

FIG. 8.

(Arlitt and Wells: Effect of alcohol on reproductive tissues.)

MENINGITIS IN AN INFANT DUE TO A HITHERTO UNDESCRIBED ORGANISM, MICROCOCCUS FLORENS.

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PLATE 61.

(Received for publication, August 1, 1917.)

The clinical and bacteriological observations which we wish to record were made upon a case of meningitis which was admitted to the Harriet Lane Home of the Johns Hopkins Hospital. The clinical history was as follows:

H. S., age 6 months (Text-fig. 1). Dec. 15, 1916, 8 p.m. Admitted to the Harriet Lane Home in convulsions which had been continuous since 6 p.m. He had been treated in the dispensary for the preceding 3 weeks for otitis media. The temperature had been between 100.8° and 101.8° F. at each visit. Pus had been obtained from both ears by myringotomy.

The convulsions began suddenly on the day of admission. The child had vomited all of his feedings, was feverish, and looked very ill. On admission to the hospital he was found to be well developed and well nourished. He was having general convulsions, most marked in the left arm and leg. The eyes were fixed, staring, and deviated to the left. The convulsions were promptly controlled by sedatives. The physical examination was negative except for the double otitis media. The temperature was 102.4°F. There was no stiffness of the neck, no hyperesthesia, and Kernig's sign was negative. A lumbar puncture was done and a purulent fluid under increased tension obtained. The cells in the fluid were composed in large part of polymorphonuclear leukocytes with a few lymphocytes and several rather conspicuous, large mononuclear phagocytic cells. These became more numerous later in the disease. No organisms were demonstrable either in cultures or films. At the time of puncture, 15 cc. of the antimeningococcus serum of the Department of Health of the City of New York were given intraspinally. On the morning following admission the temperature had fallen to 100.3°F., but the child displayed frank evidences of meningitis, marked opisthotonus, painful rigidity of the neck, painful flexion

of the spine, positive Kernig sign, tache cérébrale, and marked hyperesthesia. The fontanelle was not bulging.

Because of the purulent fluid active serum therapy was continued twice daily in spite of the constant absence of organisms in films and cultures.

Dec. 18, 3rd day after admission. No residual signs of meningitis except slight cervical rigidity. The spinal fluid was clear and no serum was given.

Dec. 21, 7th day of the disease. The temperature rose to 102.5° F. and the child was irritable. The fluid was cloudy but no organisms could be demonstrated. Serum was again given and repeated on the following day.

The thirteenth puncture on the 13th day of the disease yielded fluid which was only slightly turbid. 4 days later, Dec. 31, it was perfectly clear with but 80 cells per c.mm.

Dec. 26–Jan. 3, 1917. Temperature normal; the patient seemed perfectly well.

Jan. 3, 20th day of the disease. There was a rise of temperature to 101.5° F., the child vomited in a projectile manner, became very irritable, and the neck again became stiff and retracted. The spinal fluid was cloudy and 12 cc. more of serum were given.

Jan. 6. The temperature again became normal and remained so for the 5 following days. The one lumbar puncture (No. 17) done during this time yielded almost clear fluid which contained 500 cells per c.mm., but no demonstrable organisms.

On the 29th day of the disease there was an abrupt rise of temperature to 102.8°F. and the child displayed again all the acute signs of meningitis. The eighteenth lumbar puncture gave very cloudy fluid in which, for the first time, organisms were demonstrable in great abundance both in films and cultures. They were Gram-negative intra- and extracellular diplococci, many of them arranged in groups of twos and threes. Serum was given then and for the 5 following days.

The fluid continued to be quite purulent, often as much as 1 cc. of pus settled in the bottom of a 6 cc. tube. Organisms continued to be present in the greatest abundance.

Jan. 14, 31st day. A ventricular puncture was done because it was impossible to withdraw the thick purulent material from the lumbar spine. Purulent fluid containing many organisms was obtained. Serum was given by the same route then and for the 2 days following.

Jan. 18. A Gram-negative diplococcus, identical with that found in the spinal fluid, was isolated from the blood stream.

The child's condition gradually became worse. He was stuporous, lying in a position of marked opisthotonus (Figs. 1 and 2). He paid no attention to his surroundings, but the fundi presented a normal appearance. The child was extremely hyperesthetic. His neck was very rigid, the extremities were quite spastic, and reflexes hyperactive. The fingers were tightly flexed at the wrist. It was necessary to pad the palms to prevent the finger nails from cutting into the palms.

Although food was well taken there were frequent attacks of projectile vomiting. There were no convulsions.

The opisthotonus increased until the soles of the feet almost touched the occiput. Fluid from the ventricle and lumbar spine 3 days before death was purulent and full of organisms.

Jan. 28, 45th day of the disease. The child died after the temperature had risen to 105.8° F. Autopsy was denied.

The bacteriology of the Gram-negative micrococcus repeatedly isolated from the spinal fluid of this case is of especial interest, inasmuch as the reactions of this organism do not correspond with those heretofore described by Weichselbaum and Ghon,[1] Jaeger,[2] Gordon,[3] Dopter and Pauron,[4] von Lingelsheim and Leuchs,[5] Shennan and Ritchie,[6] Elser and Huntoon,[7] and other investigators.[8] Because of its luxuriant growth on all media tested, we have tentatively called this organism *Micrococcus florens*.

As appears from Table I, this organism was isolated from four lumbar punctures, four ventricular punctures, and one blood culture. Three of these cultures were studied extensively; namely, one from the lumbar puncture on January 15, 1917, 13 days before death; one from the ventricular puncture on January 25, 3 days before

[1] Weichselbaum, A., *Fortschr. Med.*, 1887, v, 573; *Wein. klin. Woch.*, 1905, xviii, 992. Weichselbaum, A., and Ghon, A., *Wien. klin. Woch.*, 1905, xviii, 625.

[2] Jaeger, H., *Z. Hyg. u. Infectionskrankh.*, 1895, xix, 351; Die Cerebrospinalmeningitis als Heeresseuche, Berlin, 1901; *Z. Hyg. u. Infectionskrankh.*, 1903, xliv, 225.

[3] Gordon, Great Britain National Health Insurance, Medical Research Commission, Special Report Series, No. 3, London, 1915–16.

[4] Dopter, C., *Compt. rend. Soc. biol.*, 1910, lxix, 546, 600; *Paris méd.*, 1911–12, ii, 211, 461. Dopter and Pauron, *Comp. rend. Soc. biol.*, 1914, lxxvii, 157, 231.

[5] von Lingelsheim, W., *Klin. Jahrb.*, 1906, xv, 373; *Z. Hyg. u. Infectionskrankh.*, 1908, lix, 457. von Lingelsheim and Leuchs, *Klin. Jahrb.*, 1906, xv, 489.

[6] Shennan, T., and Ritchie, W. T., *J. Path. and Bacteriol.*, 1908, xii, 456.

[7] Elser, W. J., and Huntoon, F. M., *J. Med. Research*, 1909, xx, 371.

[8] Arkwright, J. A., *J. Hyg.*, 1907, vii, 145; *Brit. Med. J.*, 1915, i, 494; *Proc. Roy. Soc. Med.*, 1914–15, viii, Sect. epidemiol. and state med., 69. Flexner, S., *J. Exp. Med.*, 1907, ix, 142; *Med. Rec.*, 1915, lxxxvii, 137. Wollstein, M., *J. Exp. Med.*, 1907, ix, 588. Du Bois, P. L., and Neal, J. B., *Am. J. Dis. Child.*, 1915, ix, 1; *J. Immunol.*, 1916, i, 307. Robinson, G. H., *J. Immunol.*, 1917, ii, 451 (also in *J. Am. Med. Assn.*, 1917, lxviii, 1660).

TABLE I.

Date.	Day of disease.	Temperature. a.m.	Temperature. p.m.	Signs of meningitis.	Lumbar puncture.	Ventricular puncture.	Character of fluid.	Organisms. Films.	Organisms. Cultures.	Amount of serum given.	Remarks.
1916		°F.	°F.							cc.	
Dec. 15	1		101.8	None.	1		Purulent.	0	0	15	White blood count 29,800.
					2			0	0		
" 16	2	100.3	101	Opisthotonus; positive Kernig sign; rigidity; hyperesthesia, etc.	3		"	0	0	10	
					4			0	0	10	
" 17	3	98.2	100	Cervical rigidity; Kernig.	5		Cloudy.	0	0	10	Von Pirquet sign negative.
					6			0	0	10	
" 18	4	98.4	98.5	Slight cervical rigidity.	7		Clear.	0	0		No tubercle bacilli found in films; globulin positive.
" 20	6	99	100	Cervical rigidity; hyperesthesia.	8		Turbid.	0	0	12	Nasal culture negative.
" 21	7	102.5	102	" "	9		"	0	0	10	Urticaria.
" 22	8	99	99	" "	10		"	0	0	15	
" 24	10	99.2	100.2	" "	11		"	0	0	10	
" 25	11	99.6	100	Bulging fontanelle; spastic extremities.	12		600 cells per c.mm.; cloudy.	0	0		
" 27	13	98.6	98.2	Less marked.	13		Less cloudy.	0	0		Urine repeatedly negative.
" 31	17	98.6	98.6	None.	14		80 cells per c.mm.; clear.	0	0		Globulin faintly positive.

1917												
Jan.	3	20	99.3	101.5	Vomiting; stiff neck; hyperesthesia.	15		Purulent.	0	0	12	
"	4	21	101.4	102	" "		1	"	0	0		Fundi normal.
"	5	22	100.4	100.8	" "	16		"	0	0		Nasal culture negative.
"	8	25	98.8	98.6	None.	17		500 cells per c.mm.; almost clear.	0	0		
"	12	29	102.8	102.4	All.	18 19		Purulent.	++	++	10	Gold chloride gives atypical meningitic curve.
"	13	30	100.3	101.7	"	20 21		"	++	++	15 10	
"	14	31	101.3	101.8	"	22	2	"	++	++	10 10	
"	15	32	102	101	"	23	3	"	++	++	10	
"	16	33	101.9	101.8	"		4	"	++	++	10	Jan. 18. Blood culture positive.
"	25	42	102.3	100	"	24	5	"	++	++	Total serum given 189 cc.	

death; and the third obtained from a ventricular puncture made January 28, 5 hours after death. All the reactions of the Gram-negative micrococci obtained from these three punctures were constant. In the last puncture, in addition to this Gram-negative micrococcus, a Gram-positive coccus which subsequently proved to be *Staphylococcus albus* was obtained. Both the Gram-negative and Gram-positive cocci were found intra- and extracellular in the postmortem ventricular fluid.

Morphologically this organism is somewhat larger than the *Diplococcus intracellularis meningitidis* of Weichselbaum, although small forms were found in many of the cultures. It occurs chiefly as a diplococcus, although after subcultures single cocci, tetrads, and often larger groups of many cocci were found. No chains were demonstrable. In films the arrangement often resembled that of staphylococci.

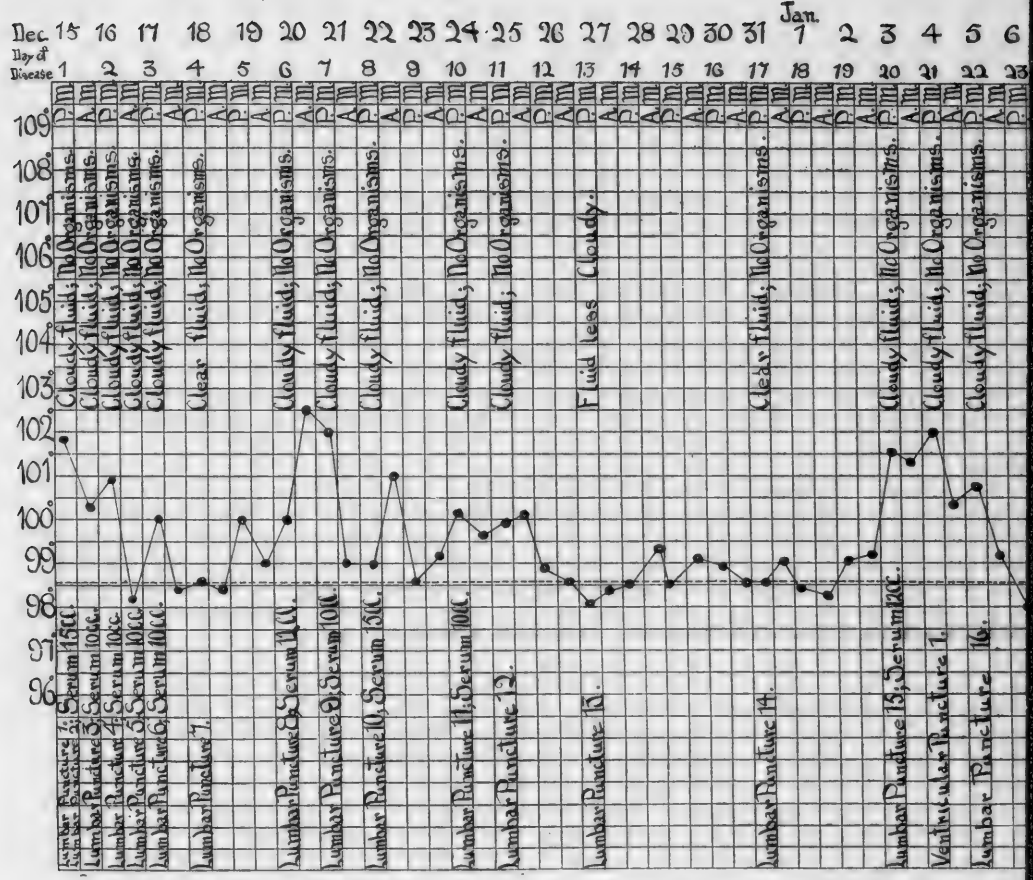
Growth was luxuriant on the surface of 2 per cent agar, serum agar, blood agar, dextrose and other carbohydrate agars, hydrocele agar, and gelatin, and in beef broth and peptone water. On gelatin no growth occurred along the stab; the growth on the surface was luxuriant. No liquefaction was produced. No growth could be obtained beneath the surface of solid media. Growth on the various media was also luxuriant at room temperature. Agar cultures remained alive for more than 48 days.

Single colonies on agar plates at the end of 16 hours were round and moist, with a smooth and uniformly elevated surface, and measured 1.5 mm. in diameter. They were grayish white in color. At the end of 4 or 5 days they became a dirty grayish brown.

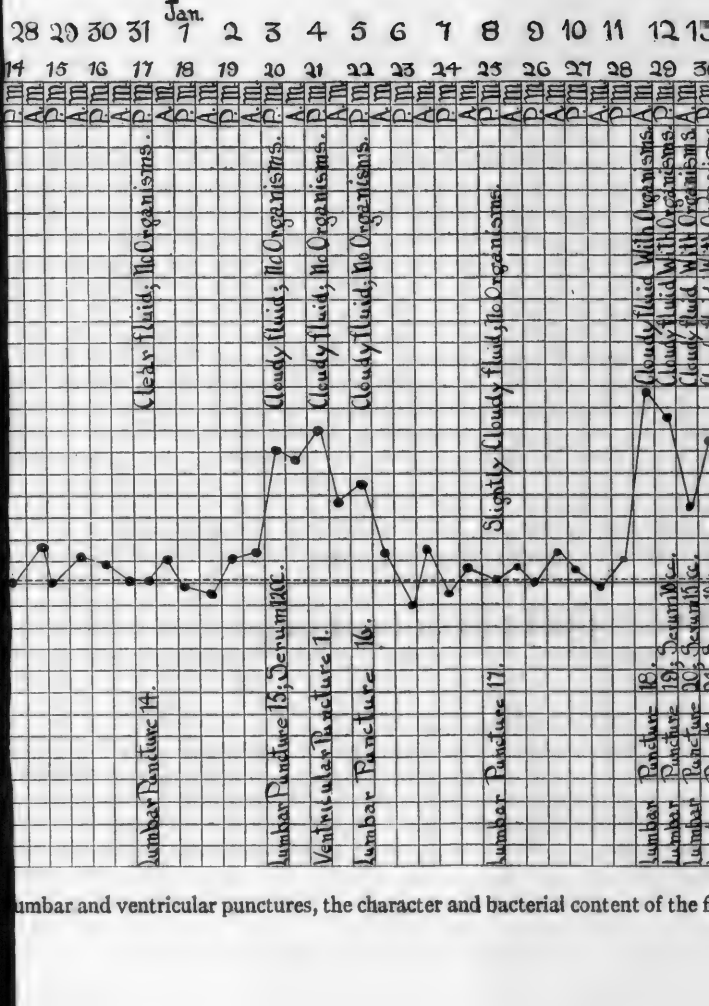
Table II shows the fermentation reactions of this organism (*Micrococcus florens*), *Bacillus typhosus*, *Micrococcus catarrhalis*, and *Bacillus fæcalis alkaligenes* grown on media from the same batch. The reactions were constant on all the media used; namely, beef broth, peptone water, and 2 per cent agar, containing 1 per cent of the various carbohydrates and 0.1 per cent of Andrade's indicator.[9]

Micrococcus florens did not produce gas in any media, but formed acid in arabinose, dextrose, and galactose. In the agar slant cultures no growth occurred below the surface.

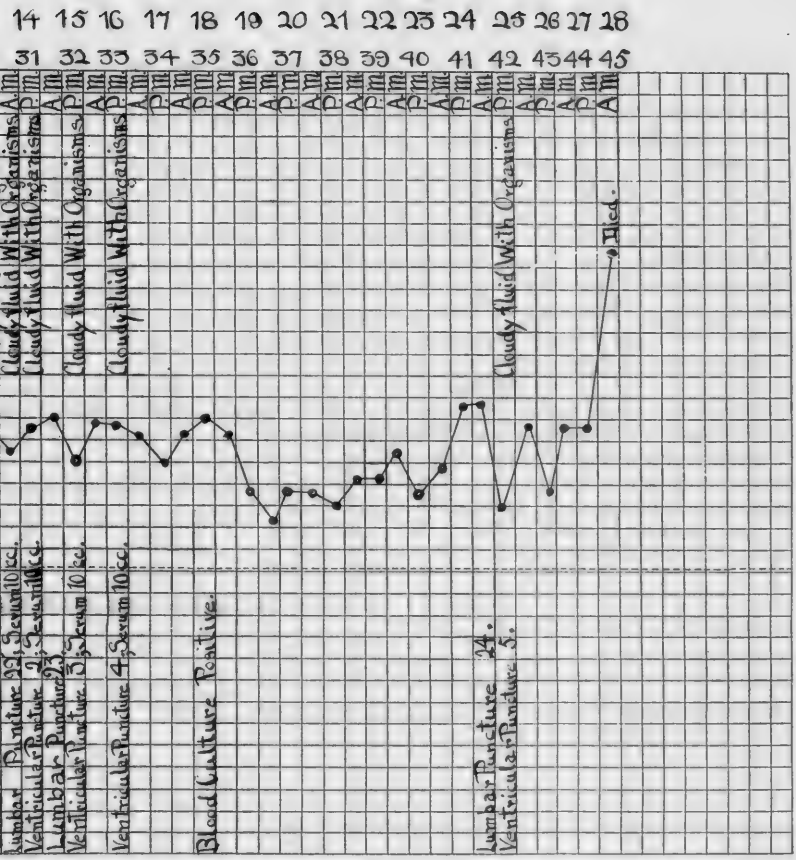
[9] Andrade, E., *J. Med. Research*, 1905–06, xiv, 551.


Dec. 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 Jan. 1 2 3 4 5 6
Day of Disease 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
A.M. P.M.
109° 108° 107° 106° 105° 104° 103° 102° 101° 100° 99° 98° 97° 96°
Cloudy Fluid; No Organisms.
Clear fluid; No Organisms.
Fluid less Cloudy.
Lumbar Puncture 1; Serum 15cc.
Lumbar Puncture 2; Serum 15cc.
Lumbar Puncture 3; Serum 10cc.
Lumbar Puncture 4; Serum 10cc.
Lumbar Puncture 5; Serum 10cc.
Lumbar Puncture 6; Serum 10cc.
Lumbar Puncture 7.
Lumbar Puncture 8; Serum 10cc.
Lumbar Puncture 9; Serum 10cc.
Lumbar Puncture 10; Serum 15cc.
Lumbar Puncture 11; Serum 10cc.
Lumbar Puncture 12.
Lumbar Puncture 13.
Lumbar Puncture 14.
Lumbar Puncture 15; Serum 12cc.
Ventricular Puncture 1.
Lumbar Puncture 16.

TEXT-FIG. 1. Temperature chart showing the relations of the lumbar and ventricular punctures, the chara


Jan.
28 29 30 31 1 2 3 4 5 6 7 8 9 10 11 12
14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29
Clear fluid; No Organisms.
Lumbar Puncture 14.
Cloudy fluid; No Organisms.
Lumbar Puncture 15; Serum 12cc.
Cloudy fluid; No Organisms.
Ventricular Puncture 1.
Cloudy fluid; No Organisms.
Lumbar Puncture 16.
Slightly Cloudy fluid; No Organisms.
Lumbar Puncture 17.
Cloudy fluid With Organisms.
Lumbar Puncture 18.
Cloudy fluid With Organisms.
Lumbar Puncture 19; Serum 16cc.
Lumbar Puncture 20; Serum 15cc.

umbar and ventricular punctures, the character and bacterial content of the f


14 15 16 17 18 19 20 21 22 23 24 25 26 27 28
31 32 33 34 35 36 37 38 39 40 41 42 43 44 45
A.m. P.m.
Cloudy Fluid With Organisms
Cloudy Fluid With Organisms
Cloudy Fluid With Organisms
Cloudy Fluid With Organisms
Cloudy Fluid With Organisms
Died.
Lumbar Puncture 2d. Serum 10cc.
Ventricular Puncture 2d. Serum 10cc.
Lumbar Puncture 3d. Serum 10cc.
Ventricular Puncture 3d. Serum 10cc.
Ventricular Puncture 4th. Serum 10cc.
Blood Culture Positive.
Lumbar Puncture 4th.
Ventricular Puncture 5th.

uid obtained, of the blood culture, and of the serum therapy to the temperature curve.

TABLE II.

Organism.	Glycerol.	Arabinose.	Dextrose.	Galactose.	Mannite.	Levulose.	Lactose.	Maltose.	Saccharose.	Raffinose.	Dextrin.	Inulin.	Salicin.
*M. florens**.....	0	+(24 hrs.)	+(24 hrs.)	+(24 hrs.)	0	0	0	0	0	0	0	0	0
M. catarrhalis.....		0	0	0									
B. fæcalis alkaligenes			0	0	0	0	0	0	0		0		
B. typhosus.....			+(24 hrs.)	+(24 hrs.)	+(24 hrs.)	+(24 hrs.)	0	+(24 hrs.)	0		+(24 hrs.)		

*These fermentation reactions were tested twelve times by two different bacteriologists. Carbohydrates of two different lots were used. Tests were incubated at 37°C. for 30 days and read daily.

The reactions of *Micrococcus florens* with dextrose and arabinose on solid media were slightly less marked than in the fluid media, but that for galactose was equally well marked on solid and in fluid media.

The media were made and sterilized in the following way: Beef broth, peptone water, and 2 per cent agar were made according to the directions recommended by Hiss and Zinsser.[10] To each of the different series 1 per cent of one of the thirteen carbohydrates was added. Merck's pure carbohydrates were used in these tests. Andrade's indicator was then added up to 0.1 per cent. A small amount of this mixture was measured into a tube, warmed, and the reaction was made neutral with normal sodium hydroxide; *i.e.*, just pink while hot. The amount of normal sodium hydroxide calculated to make the total volume of each series neutral was then added to each lot and the different series were tubed. The media were colorless when cool.

They were sterilized by placing them in an Arnold sterilizer which had first been heated to 100°C. The tubes were sterilized at this temperature for 20 minutes on 3 consecutive days. They were then incubated for 48 hours at 37°C. and proved sterile.

That this method of manufacture and sterilization of the media did not break down the various carbohydrates is demonstrated by the fact that on media of the same batch *Bacillus typhosus*, *Micrococcus catarrhalis*, and *Bacillus fæcalis alkaligenes* gave their characteristic fermentation reactions.

Serological Reactions.

The agglutination method used in these tests was that introduced by Dreyer[11] in 1904, with the exception that instead of the formolized standardized cultures used by Dreyer, living 24 hour beef broth cultures were employed except for the normal and para meningococci. For the normal and para meningococci a saline emulsion of a 24 hour serum agar slant culture was used. The reason for the

[10] Hiss, P. H., and Zinsser, H., A text-book of bacteriology, New York, 3rd edition, 1916.

[11] Dreyer, G., *J. Path. and Bacteriol.*, 1909, xiii, 331 (also Davison, W. C., *J. Am. Med. Assn.*, 1916, lxvi, 1297; *J. Lab. and Clin. Med.*, 1917, ii, 607).

latter exception was the difficulty of growing meningococci in beef broth.

The opacities of all these cultures, both saline emulsions and broth cultures, were made approximately equal, so that the results of these tests may be compared with each other.

All the agglutination tests were repeated several times on different days, and with the exception of a progressive diminution in titer of the patient's serum and spinal fluid due to age, the results of all the tests were constant. Tables III to VII show characteristic agglutination tests made on the same day.

Table III shows the results of agglutination tests made with the patient's serum, obtained 5 hours after death, and living 24 hour beef

TABLE III.

Serum.	Organism.	Dilutions of serum.				Control.
		1:25	1:50	1:125	1:250	
Patient's serum.	*M. florens.*	T.*	T.	Tr.	0	0
" "	*M. catarrhalis.*	0	0	0	0	0
" "	Normal meningococcus.	0	0	0	0	0
" "	Para "	0	0	0	0	0

* In the tables T. indicates total, or complete agglutination, the supernatant fluid being absolutely clear and all the bacteria being in the sediment at the bottom of the tube; S., standard, or medium sized flocculi in the fluid without sedimentation; and Tr., trace, or agglutination just visible to the naked eye.

broth cultures of *Micrococcus florens* and *catarrhalis*, and saline emulsions of normal and para meningococci.[12] The patient's serum agglutinated the organism we have isolated and did not affect *Micrococcus catarrhalis* or the normal or para meningococcus.

Table IV shows the results of agglutinative tests made with the patient's ventricular fluid, obtained 5 hours after death, and *Micrococcus florens*, *Micrococcus catarrhalis*, and normal and para meningococci. The results of this test are similar to, though with a lower titer than those obtained with the patient's serum.

[12] We are indebted to Dr. Harold L. Amoss and Dr. Wilson G. Smillie of The Rockefeller Institute for Medical Research for sending us these cultures of meningococci.

Table V shows the result of similar agglutination tests made with the serum from a rabbit before and after immunization with two intravenous doses of a saline emulsion of agar slants of *Micrococcus florens*. This serum, after immunization, only agglutinated cultures of *Micrococcus florens* and did not affect either *Micrococcus catarrhalis*, *Staphylococcus albus*, or the normal or para meningococcus.

Table VI shows the results of agglutination tests made with the polyvalent antimeningitis serum issued by the Department of

TABLE IV.

Serum.	Organism.	Dilutions of serum. 1:25	1:50	1:125	1:250	Control.
Patient's ventricular fluid.	*M. florens.*	Tr.	0	0	0	0
" " "	*M. catarrhalis.*	0	0	0	0	0
" " "	Normal meningococcus.	0	0	0	0	0
" " "	Para "	0	0	0	0	0

TABLE V.

Serum.	Organism.	Dilutions of serum. 1:25	1:50	1:125	1:250	1:500	1:1,250	1:2,500	Control.
Rabbit serum before immunization.	*M. florens.*	0	0	0	0	0	0	0	0
" " after "	*M.* "	T.	T.	T.	T.	T.	Tr.	0	0
" " " "	*M. catarrhalis.*	0	0	0	0	0	0	0	0
" " " "	Normal meningococcus.	0	0	0	0	0	0	0	0
" " " "	Para "	0	0	0	0	0	0	0	0
" " " "	*S. albus.*	0	0	0	0	0	0	0	0

Health of the City of New York and *Micrococcus florens*, *Micrococcus catarrhalis*, and the normal and para meningococcus. This serum agglutinated only the normal and para meningococcus emulsion and did not affect the organism of our case (*Micrococcus florens*) or *Micrococcus catarrhalis*.

Table VII shows the result of an agglutination test made with The Rockefeller Institute polyvalent antimeningitis serum and *Micrococcus florens*, *Micrococcus catarrhalis*, and the normal and para

meningococcus. The results in Table VII are similar to those in Table VI.

Table VIII shows the results of autolysis experiments.[13] The growth from 16 hour sheep serum agar slant cultures of each organism was suspended in 10 cc. of normal saline solution, seven drops of toluene being added to each tube and the tube well shaken. The tubes were incubated and examined at the end of 4 and 6 hours.

TABLE VI.

Serum.	Organism.	Dilutions of serum.					Control.
		1:25	1:50	1:125	1:250	1:500	
Polyvalent antimeningitis serum (Department of Health, New York City)	*M. florens.*	0	0	0	0	0	0
" "	*M. catarrhalis.*	0	0	0	0	0	0
" "	Normal meningococcus.	T.	T.	S.	Tr.	0	0
" "	Para "	"	"	T.	"	0	0

TABLE VII.

Serum.	Organism.	Dilutions of serum.							Control.
		1:25	1:50	1:125	1:250	1:500	1:1,250	1:2,500	
Polyvalent antimeningitis serum (Rockefeller Institute).	*M. florens.*	0	0	0	0	0	0	0	0
" "	*M. catarrhalis.*	0	0	0	0	0	0	0	0
" "	Normal meningococcus.	T.	T.	T.	T.	Tr.	0	0	0
" "	Para "	"	"	"	"	S.	Tr.	0	0

As will be seen from Table VIII *Micrococcus florens* does not autolyze as readily as the normal and para meningococcus, while *Micrococcus flavus* 3 autolyzes still less than *Micrococcus florens.*

[13] These tests were kindly made by Dr. Wilson G. Smillie of The Rockefeller Institute for Medical Research.

TABLE VIII.

Organism.	Degree of autolysis.	
	4 hrs.	6 hrs.
Normal meningococcus (Rockefeller Institute)	+++	Almost complete.
Para " " "	++	+++
M. florens	+−	+
M. flavus 3	−	−

Pathogenicity Tests.

Mice.—(*a*) and (*b*). The pathogenicity of *M. florens* for mice was tested on two animals. 1.0 cc. of a saline emulsion containing one-tenth of a living 24 hour agar slant of this organism when injected into the peritoneums of two white mice produced death in 11 to 18 hours in each instance.

Films made of the milky peritoneal exudate and stained with Hiss' capsular stain[10] failed to show any capsules. *M. florens* was recovered in cultures, made at the autopsies, of the heart's blood and of the peritoneal exudate.

Rabbits.—The pathogenicity of *Micrococcus florens* for rabbits was tested on four animals.

(*a*) 1.5 cc. of a saline emulsion containing one-sixth of a living 24 hour agar slant of the organism injected into the lumbar cord[14] of Rabbit 1 failed to cause any symptoms for 15 days. On the 15th day 1.75 cc. of a saline emulsion containing one-fifth of a living 24 hour agar slant of the organism were again injected into the lumbar cord. This injection produced death within 12 hours.

The autopsy showed slight thickening of the meninges, enlargement of the spleen, and marked injection of the blood vessels of the intestine and mesentery. Histological sections of the brain and spinal cord showed the presence of meningitis. Cultures of the spinal cord and the heart's blood, made at autopsy, showed the presence of *M. florens*.

Table IX shows the result of agglutination tests made with serum from this rabbit, obtained immediately before its first inoculation, and on the 15th day, immediately before the second inoculation. The production of agglutinins for *Micrococcus florens* is demonstrated.

(*b*) 0.5 cc. of a saline emulsion containing one-twentieth of a living 24 hour agar slant of *M. florens* was injected intracerebrally (left frontal lobe) into Rabbit 2. No symptoms were noted for 10 days. At the end of that time, 0.5 cc. of a

[14] The technique for lumbar puncture for rabbits is that employed by C. R. Austrian (*Bull. Johns Hopkins Hosp.*, 1916, xxvii, 237).

saline emulsion containing one-fifth of a living 24 hour agar slant of *M. florens* was again injected intracerebrally.

A culture of the heart's blood, made by aseptic cardiopuncture 24 hours after the last inoculation, showed a growth of *M. florens*. A progressive weakness and general malaise were noted for the next 10 days, at the end of which death occurred.

M. florens was recovered from cultures made at autopsy from the brain and spinal cord. Histological sections of the brain and spinal cord showed evidence of a chronic meningitis.

(*c*) and (*d*). 1.0 cc. of a saline emulsion containing one-fourth of a living 24 hour agar slant of *M. florens* was injected into the lower lumbar cords of Rabbits 3 and 4. No. 3 died in 8 to 14 hours. No. 4 died in 12 to 18 hours.

M. florens was recovered from cultures made at autopsy of the heart's blood and the spinal cord of each rabbit. The rabbits' sera before inoculation and after death did not agglutinate *M. florens*. Microscopic sections of the spinal cords showed the presence of an acute purulent meningitis in each animal.

TABLE IX.

Serum.	Organism.	Dilutions of serum.				
		1:25	1:50	1:125	1:250	Control.
Rabbit 1 (1st day) before first inoculation.....	*M. florens.*	0	0	0	0	0
" 1 (15th ") " second "	*M.* "	T.	Tr.	0	0	0

Monkeys.—(*a*) 0.5 cc. of saline emulsion containing one-fifth of a living 24 hour agar slant of *M. florens* was inoculated into the left frontal lobe of a monkey. This monkey, a *Macacus rhesus*, was obviously suffering from miliary tuberculosis at the time. For the next 15 hours no symptoms of meningitis could be noted. Cultures of the heart's blood, obtained by cardiopuncture, at the end of this time revealed a growth of *M. florens*.

At the end of 24 hours 0.5 cc. of a saline emulsion containing one-fifth of a living 24 hour culture of *M. florens* was again injected intracerebrally, into the left frontal lobe. At the end of 12 hours the monkey was decidedly ill and very irritable, lying on the bottom of his cage, refusing food. No opisthotonus could be noted although the monkey evidenced pain when his neck was bent. Symptoms became more marked and death followed in 60 hours after the second inoculation.

The autopsy showed miliary tuberculosis involving the mediastinal and mesenteric glands, the great omentum, the kidneys, the spleen, and the body of the tenth thoracic vertebra. No tuberculous processes were noted in the central nervous system. Films of the brain and spinal cord showed large numbers of polymorphonuclear leukocytes and Gram-negative diplococci. There were ad-

hesions between the frontal and temporal lobes. Histological sections of the brain and spinal cord gave obvious evidence of acute purulent meningitis. Cultures made of the heart's blood, brain, and spinal cord showed growths of *M. florens*.

Table X shows the results of agglutination tests made with this monkey's serum before the first inoculation and at autopsy.

(*b*) 1.5 cc. of a saline emulsion containing one-quarter of the growth from one 24 hour agar slant culture of *M. florens* were injected into the lower lumbar

TABLE X.

Serum.	Organism.	Dilutions of serum.				Control.
		1:25	1:50	1:125	1:250	
Monkey 1, before first inoculation	*M. florens.*	0	0	0	0	0
" 1, at autopsy (4 days later)	*M.* "	T.	T.	T.	T.	0

TABLE XI.

Serum.	Organism.	Dilutions of serum.				Control.
		1:25	1:50	1:125	1:250	
Monkey 2, before first inoculation	*M. florens.*	0	0	0	0	0
" 2, " third " (22 days later)	*M.* "	T.	T.	T.	T.	0
" 2, at autopsy (24th day)	*M.* "	"	"	"	"	0

cord of a healthy *Macacus rhesus* (Monkey 2). No symptoms were noted for the next 3 days. At the end of this time 1.5 cc. of a saline emulsion containing one-half of the growth from a 24 hour agar slant culture were injected into the lower lumbar cord. No symptoms occurred for the next 19 days. At the end of this time 1.75 cc. of a saline emulsion containing all the growth from one 24 hour agar slant of *M. florens* were again injected into the lower lumbar cord. Death resulted in 8 to 18 hours.

M. florens was recovered in cultures made at autopsy from the heart's blood, brain, and spinal cord. Histological sections of the brain and spinal cord showed evidence of acute purulent meningitis. No other lesions could be found.

Table XI shows the results of agglutination tests made with this monkey's serum before the first inoculation, before the third inoculation (22 days after the first and 19 days after the second inoculation), and after death.

CONCLUSIONS.

These virulence tests lead us to believe that *Micrococcus florens* is pathogenic for mice, rabbits, and monkeys (*Macacus rhesus*), producing septicemia, and that it will also cause purulent meningitis in the two latter animals.

The combined results of the biological reactions and of the agglutination tests lead us to believe that we are dealing with an organism which, though morphologically similar to the meningococcus, yet is entirely distinct.

A review of the literature fails to reveal any description of a Gram-negative micrococcus which grows luxuriantly on all media tested, forms no pigment, and ferments arabinose, dextrose, and galactose.

We wish to express our thanks to Dr. Anna Williams and to Dr. Stanhope Bayne-Jones for their valuable suggestions and assistance.

EXPLANATION OF PLATE 61.

FIGS. 1 and 2. These photographs were taken 3 days before death, showing the child in different positions. The opisthotonus was extreme.

FIG. 1.

FIG. 2.

(Davison, Davison, and Miller: Meningitis due to *Micrococcus flavens*.)

THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

XVI. The Influence of Splenectomy and of Blood Disintegration upon the Production of Bile Pigment.

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(Received for publication, July 9, 1917.)

In previous studies (1) reported from this laboratory the various phenomena which follow splenectomy in the dog have been described. In connection with the most important of these—the anemia, the increased resistance of the red cells, and the decreased tendency to jaundice—numerous indirect observations indicated that in the absence of the spleen, there is possibly a decreased formation of bile pigment. In the present work this point was studied by a direct examination of the pigment content of the bile.

Banti (2) was the first to point out that splenectomized dogs require larger doses of toluylenediamine to cause jaundice than do normal dogs. Pugliese and Luzzatti (3) confirmed this observation and brought evidence to show that the action of toluylenediamine and pyrodine upon the splenectomized animal is less marked and occurs less promptly than in the normal animal. Joannovics (4) agreed in this general opinion and claimed, as did also Vast (5), that for the destruction of the blood cells by toluylenediamine the intervention of the spleen is necessary. The only direct observations appear to be those of Pugliese (6) who states that after splenectomy the output of bile pigment in bile from a bile fistula drops to about one-half, while the other constituents of the bile are but little altered. Moreover, he showed that although after the injection of pyrodine the bile of the splenectomized dogs shows an increase in the bile pigments, the increase is not so pronounced as in the normal dog after pyrodine injection.

The recent work of Hooper and Whipple (7 *c*, *d*, *e*, *f*, *h*) does not support this claim of Pugliese. On the contrary, these observers assert that splenectomy has no constant influence on the output of bile pigments under normal conditions, though they find many interesting deviations from the normal output in splenectomized bile fistula dogs with anemia.

Methods.

The elimination of bile pigment and the changes following the administration of an hemolytic agent were studied both before and after splenectomy in dogs with a bile duct-ureter anastomosis (8); at the same time the studies were made of the red cell and hemoglobin content of the blood.

Four dogs of approximately the same size were used. They were fed on a standard diet containing 0.4 gm. of nitrogen per kilo and 70 calories per kilo of body weight. The diet consisted of beef heart, lard, bread crumbs, sugar, a little salt, and some bone ash. The daily intake of water was always the same.

The importance of keeping the test animals on a constant diet for bile pigment determination is obvious from the observations of Hooper and Whipple (7 *b*), who have shown that carbohydrates tend to increase considerably the secretion of bile pigment in dogs with bile fistula and also that on a strict meat diet the bile pigment curve is at its lowest level.

Instead of the usual external bile fistula, the bile duct-ureter fistula (or anastomosis) of Pearce and Eisenbrey (8) was employed. The method consists in diverting the bile from the intestine to the urinary bladder by anastomosing the common bile duct and the right ureter, after removal of the corresponding kidney. It has several advantages for long continued observations and avoids some of the troubles of the external fistula, such as obstruction, infection, and losses due to accidents in collecting. The mixture of urine and bile, in the absence of jaundice, offers no difficulties for the quantitative determination of bile pigments.[1]

Several days after this operation a preliminary blood examination was made and if anemia was absent, and there was no evidence of obstruction of flow of bile to the bladder, the urine was collected from the cage every morning for 1 week and the several lots were combined for quantitative estimation of bile pigment.

The animals were not catheterized, the urine being collected daily

[1] I am indebted to Professor J. E. Sweet of the Department of Surgical Research for the performance of these operations.

and preserved in cold storage until the quantitative determination of bile pigment was made.

It is well known that there are hourly and at times daily variations in bile pigment output (7 *a*, 9), and it was to avoid errors due to these variations that collections were continued for so long a time. This control period we hereafter refer to as Period A.

In the second period, of equal duration with A, Period B, an hemolytic agent, toluylenediamine, was given. This was made up in a 2 per cent solution and administered by stomach tube daily at the same hour for 7 days.

After Period B had been completed, an interval of more than 10 days was allowed to elapse to permit recovery from the anemia due to toluylenediamine. Splenectomy was then performed under ether anesthesia. 1 week after this operation, observations similar to those of Period A were made. This period is known as Period C.

In Period D, toluylenediamine was given as in Period B. In each period observations were made of the condition of the blood and of the weight and general condition. At the termination of each experiment the anastomosis, the bile passages, liver, ureter, and urinary bladder were carefully examined. No abnormalities were found except a slight dilatation of the bile ducts with slight bile stasis in the liver in Dog 2.

In the first experiment (Dog 1) two observations were made in Period A, and in the first and second (Dogs 1 and 2) two sets in Period B. In the other animals, only one set was made in all periods.

For the determinations of the amount of bile pigment in the urine, the method of Hooper and Whipple (7*a*) was used. Exactly 10 cc. of bile-urine mixture were placed in a large glass tube, 20 cc. of 10 per cent calcium chloride solution were added; the mixture was then made weakly alkaline with a saturated solution of sodium carbonate and centrifuged for 30 minutes at a speed of 1,500 revolutions per minute. After this, a small amount of calcium chloride solution was added to see whether precipitation would occur again. After complete precipitation was assured, the precipitate was washed four times with distilled water by use of the centrifuge. To dissolve the precipitate finally, the following solution was used:

	cc.
Ethyl alcohol, 95 per cent	100
Nitric acid, 50 " "	0.4
Hydrochloric acid, 37 " "	5.0

After standing for 16 to 18 hours, when the intensity of color was at its maximum, the pigment was estimated by the Duboscq colorimeter. The standardization of the wedge in the colorimeter was made against Kahlbaum's chemically pure bilirubin.

For determining the hemoglobin content of the blood the Fleischl-Miescher apparatus was used.

The resistance of the erythrocytes was examined by hypotonic salt solution; there was very slight decrease after the administration of toluylenediamine and very slight increase after the splenectomy.

RESULTS.

The general outcome of these experiments is shown in Tables I to IV. In the first period the general condition of the animal—weight and blood picture—was satisfactory. In Period B the blood changes due to toluylenediamine were accompanied by a slight loss of weight and this was evident also in Period D, but in general the changes were not sufficient to prevent comparable observations in the various periods. It should be emphasized that in the four experiments reproduced in tabular form, there was no loss of bile through faulty anastomosis and no evidence of jaundice due to obstruction. In studying the tables Period A should be compared with Period C and Period B with Period D, the former showing the effect of splenectomy alone and the latter of splenectomy on the formation of bile pigment in the presence of excessive blood destruction. This comparison shows that in each experiment the elimination of bile pigment after splenectomy was reduced and the observations are thus in accord with those of Austin and Pepper (1 *l*), which indicated that the path of the blood to the liver is an important factor in the production of jaundice. There is, however, another possible factor. Pearce, Austin, and Musser (1 *c*) have shown that the production of jaundice is more difficult in an anemic animal than in the plethoric animal, and Hooper and Whipple (7 *g*), who found that splenectomy did not influence the output of bile pigment in the dog after several months following splenectomy, describe many curious results in the anemic splenectomized animal. In all the experiments the blood picture was never so satisfactory in Period C as in Period A and the factors

of anemia and blood regeneration undoubtedly have something to do with the results we obtained. There is, however, no question, under the condition of these experiments, of the decreased elimination of bile pigment after splenectomy. In this connection it should be pointed out that the periods of continuous observation—7 days—are larger

TABLE I.

Dog 1. Weight 9.3 kilos.
Put on diet* January 15, 1917.
Bile duct-ureter anastomosis January 19.

Period.	Dose of 2 per cent toluylenediamine.	Bile pigment per day.	Blood.			Weight.
			Date.	Hemoglobin.	Erythrocytes per cm.	
1917		*gm.*	*1917*	*per cent*		*kg.*
Period A.						
Jan. 23–29		0.0678	Jan. 23	98	6,210,000	9.5
" 30–Feb. 5		0.0644	" 30	100	6,030,000	9.4
Period B.						
Feb. 6–12	10 cc. every day.	0.0698	Feb. 8	82	5,450,000	9.0
			" 13	94	5,700,000	7.8
" 21–27	13 cc. on Feb. 21, 22, 23;	0.310	" 19	103	5,170,000	
	15 cc. on Feb. 24, 25,		" 23	95	5,200,000	8.0
	26, 27.		" 27	43	4,190,000	7.8
Mar. 16. Splenectomy.			Mar. 15	101		
Period C.		0.0450	Mar. 22	89	5,300,000	8.2
Mar. 22–28			" 28	82	5,360,000	8.1
Period D.	13 cc. on Mar. 29, 30, 31;	0.208	Mar. 30	74	5,230,000	7.5
Mar. 29–Apr. 4	15 cc. on Apr. 1, 2, 3, 4.		Apr. 4	63	3,600,000	7.5

* Diet consisted of beef heart 150 gm., lard 30 gm., sugar 40 gm., bread crumbs 50 gm., bone ash 10 gm., salt 2 gm., and water 300 cc.

than those of other observers and thus obviate error due to temporary fluctuations—possibly a source of error in shorter periods of observations.

A comparison of Periods A and B shows that small doses over several days of an hemolytic poison may not cause an increase in bile pigment elimination, but if continued in larger doses or for a longer period a

fivefold increase in elimination may occur (Table I). This is in accord with the views of Pearce, Austin, and Eisenbrey (1 *b*) on the relation of hemoglobinemia to jaundice.

If Periods B and D are compared, it is seen that although in Period D an increase of pigment elimination follows blood destruc-

TABLE II.

Dog 2. Weight 10.5 kilos.
Put on diet* January 20, 1917.
Bile duct-ureter anastomosis January 23.

Period.	Dose of 2 per cent toluylenediamine.	Bile pigment per day.	Blood.			Weight.
			Date.	Hemoglobin.	Erythrocytes per cm.	
1917		*gm.*	*1917*	*per cent*		*kg.*
Period A. Jan. 30–Feb. 5		0.0622	Feb. 5	101	5,680,000	9.9
Period B. Feb. 6–12	10 cc. every day.	0.0672	Feb. 8	68	3,950,000	9.4
			" 13	71	4,010,000	8.9
			" 19	86	5,480,000	
" 21–27	13 cc. on Feb. 21, 22, 23; 15 cc. on Feb. 24, 25, 26, 27.	0.1280	" 23	52	3,550,000	8.9
			" 27	38	3,520,000	8.6
Mar. 16. Splenectomy.			Mar. 15	99		
Period C. Mar. 22–28		0.036	Mar. 22	81	4,460,000	8.9
			" 28	82	4,280,000	8.2
Period D. Mar. 29–Apr. 4	13 cc. on Mar. 29, 30, 31; 15 cc. on Apr. 1, 2, 3, 4.	0.103	Mar. 30	62	3,510,000	8.0
			Apr. 4	35	3,140,000	7.9

* Diet consisted of beef heart 150 gm., lard 40 gm., sugar 40 gm., bread crumbs 50 gm., bone ash 10 gm., salt 2 gm., and water 300 cc.

tion, this increase does not reach the high level obtained before splenectomy (Period B). There is here no question of the influence of anemia, as was the case in comparing Periods A and C, for in all experiments in Periods B and D anemia was present, and was of approximately the same degree. It is difficult therefore to avoid the conclusion that the lowered elimination of bile pigment is due to the absence of the spleen.

TABLE III.

Dog 3. Weight 8.6 kilos.
Put on diet* February 27, 1917.
Bile duct-ureter anastomosis March 1.

Period.	Dose of 2 per cent toluylenediamine.	Bile pigment per day.	Blood.			Weight.
			Date.	Hemoglobin.	Erythrocytes per cm.	
1917		*gm.*	*1917*	*per cent*		*kg.*
Period A. Mar. 5–11		0.0624	Mar. 6	102	6,120,000	8.6
Period B. Mar. 12–18	14 cc. every day.	0.288	Mar. 13	82	3,930,000	8.4
			" 18	45	3,420,000	7.6
Mar. 30. Splenectomy.			Mar. 29	100		
Period C. Apr. 5–11		0.0396	Apr. 6	82	5,120,000	8.4
			" 11	64	4,860,000	7.8
Period D. Apr. 12–18	14 cc. every day.	0.202	Apr. 13	58	3,570,000	7.5
			" 18	40	3,240,000	7.3

* Diet consisted of beef heart 110 gm., lard 35 gm., sugar 35 gm., bread crumbs 30 gm., bone ash 10 gm., salt 2 gm., and water 300 cc.

TABLE IV.

Dog 4. Weight 8.4 kilos.
Put on diet * February 27, 1917.
Bile duct-ureter anastomosis March 1.

Period.	Dose of 2 per cent toluylenediamine.	Bile pigment per day.	Blood.			Weight.
			Date.	Hemoglobin.	Erythrocytes per cm.	
1917		*gm.*	*1917*	*per cent*		*kg.*
Period A. Mar. 5–11		0.0618	Mar. 6	94	5,980,000	8.2
Period B. Mar. 12–18	14 cc. every day.	0.186	Mar. 13	77	4,360,000	8.1
			" 18	42	3,433,000	7.4
Mar. 30. Splenectomy.			Mar. 29	90		
Period C. Apr. 5–11		0.036	Apr. 6	84	4,820,000	8.1
			" 11	69	4,250,000	7.5
Period D. Apr. 12–18	14 cc. every day.	0.1256	Apr. 13	61	4,060,000	7.0
			" 18	38	3,220,000	7.2

* Diet consisted of beef heart 110 gm., lard 35 gm., sugar 35 gm., bread crumbs 30 gm., bone ash 10 gm., salt 2 gm., and water 300 cc.

SUMMARY.

1. In four animals with a bile duct-ureter anastomosis and without disturbance due to obstruction or absorption, the total quantity of bile pigment output during a day under normal conditions varied from 0.0618 to 0.0678 gm. These figures are practically identical with those of Stadelmann (9, 10) but lower than those given by Hooper and Whipple (7), who find that the average bile pigment excretion amounts to about 1 mg. per pound of body weight per 6 hours.

2. In all the experiments there is definite evidence of a decrease in bile pigment elimination after splenectomy. This is true not only of the elimination when no hemolytic agent is administered but also when excessive blood destruction is caused. Under the latter circumstances the amount of bile pigment is greatly increased but never reaches the high level of blood destruction before splenectomy.

3. These observations appear to show conclusively that the absence of the spleen influences the formation of bile pigment. To what extent the influence is mechanical, *i.e.*, change in the course of the blood to the liver, and to what extent due to anemia, cannot be stated at present.

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STAGGERS IN SHEEP IN PATAGONIA.*

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INTRODUCTION.

During the past few years a nervous disorder of sheep has become more prevalent throughout portions of Patagonia. This disorder has been given a number of names; among the more common may be mentioned staggers, temblique, loco, and huecù. From present reports the disease seems to be widespread, for it exists throughout the pampa at least as far north as the Chubut Valley and extends southward to Deseado and from the eastern boundary of the pampa to the Andes. The incidence varies greatly with the condition of the food supply; when there is a liberal amount of grass the actual number of cases is small. After a long continued drought when the fine grass supply is short, the number of sick animals is large. The mortality varies considerably, young sheep seeming to suffer most. The disease is not confined to the ovine species alone; horses and cattle succumb readily to it.

Acosta, in his publications on huecù,[1] discusses in detail the geographical distribution, symptoms, diagnosis, and prognosis of a disease similar to staggers.

* The field expenses of this investigation were borne by a sheep-farming company of London, which has not authorized the publication of its connection with the investigation, but which gave every facility for the work on its estancia in Patagonia. Here a small bunk house close to the settlement was converted into a temporary laboratory. Only the most necessary equipment and supplies were transported from New York.

[1] Acosta, J. L., Producción experimental de una enfermedad de tipo nervioso, *Rev. Zoot.*, 1914, v, 3; El "Huecù" o "Huaicù." Enfermedad de tipo nervioso propia de los herbìoros de la Patagonia, Tesis Universidad Nacional de Buenos Aires, No. 86, 1914.

His experimental feedings of a coarse tuft pampa grass (*Poa denudata*) to a horse, a bullock, and two sheep resulted in the production of definite nervous symptoms. Acosta's experiments were not controlled and inoculations were not attempted. He states that symptoms develop from 12 to 24 hours after the first feeding.

Conversation with a number of ranchers has given us some valuable data. The manager of an estancia in Chubut Territory supplied us with many interesting facts concerning staggers. On this particular farm about 1,000 sheep are pastured on a square league (9 square miles). The estancia consists largely of pampa[2] broken by many deep valleys. The water supply is excellent and consists of fresh springs coming from the hillsides close to the valley bottoms. There are many brackish water-holes as well. The herbage comprises several kinds of fine grass, two kinds of edible bush, and two coarse grasses, as well as several species of thorny bushes and a number of cacti.

Sheep were brought to this land in 1897. The land was at first unfenced and the sheep were herded. Lambs after weaning could be turned on virgin pasture. Even early in the history of the farm, employees would occasionally report a case of staggers, but it was only after the land had been fenced and animals permitted to feed over it for several years that the disease really became a menace. Beginning in 1910 more or less serious outbreaks of the disease have occurred annually. It affects lambs and hoggets (sheep under 1 year old), although adult sheep suffer to some extent. As a rule, the ewes lamb in August and the lambs are weaned in January and are then removed to separate paddocks. Early in February the malady may become epidemic and it may continue during the ensuing months until September. The incidence varies considerably with the general conditions of the pasture. When the seasons have been unusually dry and the herbage is sparse it is not unusual to see lambs affected at marking time.[3] The disease often exists at shearing in December. Under these conditions the nourishment has been insufficient since birth and the young have been forced to feed on grasses otherwise unnecessary.

[2] The term pampa in Patagonia is applied to the sandy plain beginning in the foot-hills of the Andes and sloping eastward toward the Atlantic Ocean. The vegetation of these plains is much sparser than that of those to the north.

[3] When lambs are 6 weeks old they are docked, marked, and castrated.

When there has been plenty of moisture and the grasses are sufficient, cases of staggers are not usually seen, either at lamb-marking or shearing, but a few cases may be observed in March and April. In extremely bad seasons the incidence may reach 100 per cent in certain paddocks. At this particular estancia the average mortality is about 5 per cent but in one paddock it reached 25 per cent.

In horses and cattle, especially in the wild herds, the losses from staggers are severe. Foals and calves from 4 to 9 months old are especially susceptible. In all paddocks located on the pampa the disease abounds. There are a few low lying paddocks, 200 or 300 feet lower than the pampa, where the disease has never existed. Sheep farmers whose lands occupy the low belt of land between the pampa and the sea declare that staggers is unknown in their flocks, although only a five strand wire fence may separate them from heavily infected pastures.

A sheep farmer whose estancia was located at Lago San Martin, about 250 leagues southwest of Camarones, informed us that his land would support 3,000 sheep to the league. Sheep had been grazed on the land for 7 years. During the first years staggers did not exist, but as the grasses became more sparse staggers became prevalent although not alarming. He stated that the young animals were affected shortly after weaning. The greatest number of cases occurred in the paddocks close to the settlement when large numbers of sheep were pastured temporarily during shearing and dipping. The fine grass in these paddocks had become very close. When questioned about the prevalence of a certain grass he informed us that it grew in all of his paddocks except those that had an elevation of over 1,500 feet. The average elevation of the paddocks was about 900 feet. Further questioning brought out the important point that he had never seen the grass commonly called pampa or coiron growing on the pampa below Deseado.

Description of the Disease.

At first sight the animal may appear normal. Excitement becomes great when the individual is alarmed by the barking of a dog, voices, etc. On becoming frightened it stands with a wild, excited look. The

neck is extended and there is usually a marked trembling of the head. Muscular twitchings of the hind legs are a constant symptom; if the animal is driven it usually breaks into a panic-stricken run. After running a short distance, stiffness of the limbs becomes marked. This stiffness is usually more noticeable in the hind legs. It moves with short, convulsive strides, and suddenly plunges forward, falling with the hind legs extended backward, and often rolls on its side (Figs. 1 to 4). If the sheep falls on a hillside it is not unusual for it to roll over and over until a level plain is reached. It is quite common to observe a sudden stiffness of all four legs; when this occurs the individual may fall directly on its side. When the animal falls it displays extreme excitability; the eyes bulge and the pupils are dilated. The head is drawn back, the muscles of the neck are tense, and the legs are extended rigidly from the body, with the digits spread far apart. If a sharp sound is made the muscles become more rigid. If the sheep is permitted to lie undisturbed the muscles gradually become flaccid, and the animal rises to its feet with some difficulty and moves away, stiffly at first, but the gait soon becomes normal. If it is frightened the same phenomena are repeated. Often there is an impairment of vision; animals may shy away from fences or attempt to jump over objects which are at a considerable distance.

When an animal has fallen and through weakness is unable to rise, it is usually found lying on its side (Fig. 5). At the slightest alarm it will make peculiar cantering movements of the legs until seized by the usual convulsions. It is not unusual to see a considerable excavation which has been made by the feet of such an animal. Often they are killed or mutilated by birds or carnivora.

Even in advanced cases of the disease, the temperature remains normal. The pulse is usually regular and weak. A ramiform congestion of the vessels of the conjunctiva is ordinarily present and the conjunctivitis is accompanied by a mucopurulent exudate. The sick animals eat well when the opportunity is afforded. There is no diarrhea or constipation.

Morbid Anatomy.

Macroscopic.—The following autopsy notes reveal the usual type of lesions found:

Subject.—Yearling hogget; wether; had been down at least 2 weeks. Pulse 68; temperature 101°F.; respirations 21. Blood: hemoglobin 90 per cent; red cells 10,608,000; leukocytes 11,500. Animal killed by chloroform; much emaciated.

Heart.—The pericardium appears normal. The heart muscle is apparently normal. The right auricle and the right ventricle contain large dark red blood clots. The left ventricle contains a smaller clot. All the valves are normal.

Lungs.—Right: On the surface of the middle lobe is a dark purplish red discoloration measuring 9 by 6 cm., rather clearly demarcated from the usual bright pink lung tissue. On section it is found to involve the pleura and invade the subpleural tissue for a distance of 0.1 cm. The rest of the lung is normal. Left: Scattered over the posterior lobe of the left lung are many irregular, raised, indistinct, grayish white areas varying in diameter from 0.1 to 2 cm. They are discrete and crepitate on pressure.

Liver.—The liver is dark red in color and somewhat firm. On section the color is dark red. The consistency appears normal. The gall bladder is filled with dark bile.

Spleen.—Apparently normal.

Kidneys.—Right: Normal in size and reddish gray in color when viewed through the capsule, which peels off readily. On section the consistency is normal. The cortex and medulla are congested. Left: The left kidney presents the same picture as the right.

Pancreas.—The pancreas is slightly congested but otherwise normal.

Esophagus and Stomachs.—All appear normal.

Duodenum.—The vessels of the duodenum are congested. The mucosa is congested. The content is largely composed of mucus. The ileum, jejunum, cecum, and colon appear normal.

Brain.—The vessels of the dura mater are slightly congested. The superficial vessels of the cerebrum, cerebellum, and medulla are all highly congested. Along the anterior border of the cerebrum and extending 1.5 cm. on both sides of the median fissure is a triangular smoke-colored discoloration which is superficial and extends backward, reaching an apex 2 cm. from the anterior border. On the ventral aspect of the cerebrum, almost exactly opposite the dorsal

area, is another irregular patch of the same color, which measures 2 by 1.3 cm.[4] On section the deeper vessels are congested.

The cerebrospinal fluid is pinkish gray in color. The spinal cord is slightly congested. The sciatic nerve appears normal.

In most animals autopsied the lesions were of about the same type. It is unusual to observe congestion of the duodenum. In a few individuals small hemorrhages had occurred about the superficial vessels of the brain. The cerebrospinal fluid varied from straw color to pinkish gray. In all cases there was a more or less marked congestion of the kidneys with a certain amount of granular degeneration of the cortex, and, in some instances, of the medulla.

The intestinal tract was always examined for worms. In only two instances were they found and then only in small numbers. Examination of the frontal and nasal sinuses usually revealed varying numbers of the larvæ of *Œstrus ovis*. These were found as frequently in normal sheep as in those suffering with staggers.

Small pieces of muscle from the fore and hind legs and from the diaphragm were examined with M'Gowan's method for the presence of Sarcosporidia.[5] They were present in only two samples and then in very slight numbers. Frozen section of these muscles failed to reveal the parasites to any great extent.

Microscopic.—Pieces of various organs from normal sheep and those suffering from spontaneous and experimental staggers were fixed in Zenker's fluid. The sections were stained with methylene blue and eosin. The changes noted are slight and not specific.

The cardiac and skeletal muscles contain Sarcosporidia in all classes of sheep examined.

The liver as a rule appears normal. The kidneys are usually pathologic. The lesions vary considerably from congestion to cloudy swelling. The cortex is usually the seat of the processes.

The brain is generally congested throughout. The meninges appear normal except for a submeningeal black pigment.[4] The nerve cells

[4] At first these discolorations were regarded as possible lesions of staggers, but they were found in normal brains as frequently as in the brains of sick animals.

[5] M'Gowan, J. P., Investigations into the disease of sheep called "Scrapie," with especial reference to its association with sarcosporidiosis, *Edinburgh and E. Scotland Agric. Rep.*, 1914.

fail to show degenerative changes when stained with methylene blue. Congestion of the spinal cord is usually noted. In several instances small hemorrhages were visible within the gray matter. Too much stress cannot be placed on them, however, as the animals were usually killed by bleeding from the jugular veins and carotid arteries.

The spleen, lymph glands, lungs and trachea, pancreas, and adrenals fail to reveal abnormalities.

Bacteriological Findings.—Although inoculations from the internal organs and the central nervous system were made into various media and incubated both aerobically and anaerobically, in the main the cultures remained sterile. In animals that had been down for indefinite periods it was not uncommon to find several species of cocci in the brain, spinal cord, and cerebrospinal fluid.

Great care was taken in obtaining portions of the brain for cultivation. The skin was dissected away and the skull flamed with a gasoline blow-torch. A large sterile trephine was used for drilling the skull. Various portions of the brain were removed through the trephined holes. The cerebrospinal fluid was drawn immediately into sterile pipettes. Usually 15 cm. of the spinal cord were removed for inoculation into various media.

Films from the internal organs, brain, and spinal cord were prepared and stained with various aniline dyes. Bacteria were not uniformly present in any of them.

EXPERIMENTAL.

In order to ascertain whether this disease was transmissible from one animal to another, a series of experiments was undertaken. Although indications in the field seemed to point to some other etiological factor than microparasites, we undertook to establish this point definitely.

Our first series of five experiments consisted in attempts to transmit the disease by natural means and by inoculation.

Experiments 1 to 5.—Susceptible sheep were permitted to pasture with those suffering from staggers. The normal sheep remained so and those affected with the disease completely recovered.

Five yearling sheep and two young lambs were inoculated with normal salt

solution suspensions prepared from the central nervous system, viscera, and blood of sheep suffering from advanced staggers. The experimental animals were inoculated in various ways, some intraperitoneally, others intravenously, subdurally, and directly into the cerebral substance. The latter died promptly from shock. Chloroform anesthesia was resorted to when inoculations were made beneath the dura mater and into the brain substance. In a few of the inoculated individuals, considerable doses of the suspensions were introduced into the rumen with the aid of a stomach tube.

None of the inoculated animals developed suggestive symptoms. All remained healthy during our observation of over 2½ months. Adequate controls which were maintained throughout this series of experiments remained healthy.

Ten sheep from an outlying estancia on which staggers did not exist were purchased for experimental purposes. These animals were transported to the settlement and placed directly in disinfected pens. Some of these were used in the inoculation experiments and the others in the experimental feedings later.

Guinea pigs weighing 300 gm. were also inoculated with tissue suspensions from several cases of staggers. They remained well.

From the foregoing series of experiments it seemed well established that the disease could not be produced by permitting sick animals to come in contact with healthy ones. Moreover, in every instance when healthy susceptible sheep were inoculated with material obtained from advanced cases of staggers they failed to develop the disease.

Among certain individuals throughout the district, factors seemed to point to a grass as a possible cause for the disorder. The grass is commonly called coiron or pampa grass. Previously we have mentioned that staggers had not affected animals on low camps. The disease is confined to the pampa. It is well known that a coarse tuft grass grows in large quantities on the pampa at altitudes from 500 to 1,500 feet. The tufts may extend well down the sides of the valleys but they do not grow in the valley bottoms except close to the pampa where the canyons are shallow. It grows in tufts varying from 15 to 60 cm. in diameter. The height varies with the amount of moisture. Where the tufts have not been disturbed it may reach a height of 40 or 50 cm.[6]

[6] The writers wish to acknowledge their indebtedness to Dr. A. S. Hitchcock, Agrostologist of the Bureau of Plant Industry of the United States Department of Agriculture, who has identified this grass as *Poa argentina*.

A series of experiments was started to test the effect of this grass when fed to normal sheep and to those that had recently recovered from staggers.

Experiment 6.—Sept. 16, 1916. Four native yearling hoggets were placed in two small pens and fed on pampa grass. Four other animals of the same age and from the same flock were fed on alfalfa hay and served as controls. The water supply was the same in all the pens. The following notes were made during the experiment:

Sept. 20. Sheep 1 shows muscular trembling. Sept. 25. Sheep 1 is very excitable; marked weakness of hind legs; falls on becoming violently excited. Sept. 30. More violent symptoms exhibited by No. 1. Sheep 2 and 3 are highly excitable. Oct. 3. Sheep 1, 2, and 3 show characteristic symptoms of staggers; *i.e.*, short, jerky movements of the head from side to side. Sheep 1 on becoming excited loses use of the hind legs, crawls about with the fore legs, and drags the hind limbs (Fig. 6). Oct. 7. Sheep 4 shows slight shaking of the head, grinds the teeth, and has the characteristic stupid facial expression. Sheep 2 has become worse rapidly, showing the same symptoms as No. 1.

The notes of a complete physical examination of No. 1 were as follows (Fig. 6):

Sheep 1.—Half bred ewe; age 1 year. Muscular trembling, incoordination of the muscles of the hind legs, grinding of the jaws, constant defecation of hard feces during examination. The animal is slightly excited and restless. Temperature 104.8°F.; pulse 93, regular, full, and weak; respirations 116 (very warm day), rapid, shallow, and regular. The heart appears normal. The skin is loose and normal, the coat of wool excellent. The face is symmetrical with no evidence of inflammation of the lips, nostrils, or ears. There is a ramiform congestion of the conjunctiva accompanied with a mucopurulent exudate. The abdomen is distended. Micturition is frequent. Mental excitement is marked but the sensibility is normal. Muscular tremblings are constant and marked disturbances of the muscular sense are observed. The reflexes are normal.

The animal lies quietly during the examination. When assisted to rise and compelled to move, it trembles violently and appears weak. The hind legs are stiff and the fore legs are braced far apart.

Blood: hemoglobin 85 per cent; red cells 9,304,000; leukocytes 7,400.

Oct. 8. Animal 1 fell and was unable to rise. Oct. 10. Chloroformed.

Autopsy.—General condition good.

Heart.—The pericardium and heart appear normal.

Lungs.—Right, normal; left, contains one darkened area in the ventral lobe measuring 9 by 5 cm. It is clearly demarcated from the rest of the normal tissue. The lobe is somewhat congested.

Spleen.—Normal in size and consistency.

Liver.—Normal in size; the color is light brownish pink and the consistency firm. Superficially the intralobular markings are distinct. When cut the

liver appears firm and the lobules stand out clearly. The gall bladder is filled with bile.

Kidneys.—Both appear congested, otherwise normal. The bladder is empty. The suprarenals are normal.

Pancreas.—Apparently normal.

Stomachs.—All contain more or less partially digested grass; no evidence of inflammation.

Intestines.—The bowels appear normal until the jejunum is reached. Here the vessels are congested. The mucosa contains a few hemorrhagic areas varying in size from 0.2 to 0.4 cm.

Ovaries.—Apparently normal.

Uterus.—Contains a large well developed fetus.

Brain.—The meninges appear normal. The superficial vessels of the brain are engorged with blood. There is a grayish black discoloration on the anterior portion of both lobes of the cerebrum. This discoloration begins a distance of 2 cm. posterior to the anterior border and extends forward and downward to the ventral aspect. It is clearly demarcated from the rest of the tissue and lies only on the surface. The cerebellum and medulla appear normal. There is a normal amount of clear straw-colored cerebrospinal fluid. The superficial vessels of the spinal cord are congested.

Bone Marrow.—The bone marrow of the humerus and the femur appear normal. The marrow of the shaft is a dull pink color and of a stiff gelatinous consistency. That of the heads is spongy and bright red in color.

In certain tubes of the media inoculated with small pieces of brain, cocci appeared after incubation. All inoculations from the heart and liver remained sterile. Salt solution suspensions of various organs of this animal were used to inoculate the sheep employed in Experiment 5.

The other animals in the experiment gradually became worse, all developing symptoms of advanced staggers, finally falling down and becoming unable to rise.

Oct. 25. Sheep 3 fell down. Nov. 7. Sheep 2 and 4 unable to rise.

The autopsies of these animals failed to show any more characteristic lesions than those found in Sheep 1. Although careful examinations of the intestines and stomachs were made, intestinal worms were not found. A common occurrence among the sheep autopsied was the presence of the larvæ of *Œstrus ovis* in the frontal sinuses.

In all these animals examinations of the muscles with M'Gowan's method for the presence of Sarcosporidia were made. In one or two instances they were present, but in very small numbers.

Unfortunately the preceding experiment was carried out with native stock. Doubtless these animals had been exposed to the disease. The results seemed to justify a repetition of the experiment with the substitution of animals which had never been exposed to staggers.

Experiment 7.—Oct. 5, 1916. The sheep used were of the same lot as those described under the inoculation experiments. Adequate controls were kept.

Within 3 days after starting to feed the grass one animal exhibited suspicious symptoms. 2 days later two animals revealed characteristic symptoms of staggers. The other two sheep developed staggers 10 and 15 days respectively after the feeding had been begun.

One animal (No. 5) after showing distinct symptoms of staggers was unable to maintain itself on the grass and was therefore killed. The others became progressively worse, falling down at the slightest excitement (Figs. 7 and 8).

The following results were noted when the urine of the three surviving animals was examined.

Sheep 6.—Amount, 10 cc.; reaction alkaline; color golden yellow, turbid; albumin present; sugar, bile, blood, and casts absent.

Sheep 7.—Amount 8 cc.; reaction alkaline; color golden yellow, turbid; albumin present; sugar, bile, blood, and casts absent.

Sheep 8.—Amount 8.5 cc.; reaction alkaline; color golden yellow, clear; albumin present; sugar, bile, blood, and casts absent.

All the animals were finally autopsied but failed to reveal more characteristic changes than those recorded.

In Experiments 6 and 7 the pulse, respiratory rate, and temperature of each animal were recorded before and during the experiments. The temperatures varied slightly and the rate of the pulse and respiration remained practically the same.

Experiment 8.—Nov. 7, 1916. In Experiments 6 and 7 the grass used was gathered from a paddock and stored in large bags until ready to feed. The grass tufts were cut close to the bottom and the whole tussock was used. A large proportion of the tufts consist of a dry, dead center surrounded by a more or less profuse belt of green freshly growing grass. Although we had never seen sheep eat this dry center, nevertheless it was believed by many that the dry portions were responsible for the disorder. To prove definitely whether this was responsible for the malady we determined to feed only green grass from young tussocks. In the young tufts the whole mass is green and only begins to dry as the seed ripens. Every morning 5 kilos of this fresh green grass were gathered and fed to two sheep of the same lot as those used in Experiment 7. Two animals fed on alfalfa in an adjoining pen served as controls.

After 2 days' feeding on the grass both animals showed suspicious symptoms; *i.e.*, twitching of the muscles of the hind limbs, slight shaking of the head, and twitching of the ears. At the end of 1 week's feeding they had developed severe symptoms of staggers (Fig. 9). 14 days after feeding had been begun both animals fell on the slightest excitement.

Examination of the urine gave the following results:

Sheep 9.—Amount 45 cc.; reaction acid; color golden yellow, clear; specific gravity 1,060; albumin present; sugar, blood, bile, and casts absent.

Sheep 10.—Amount 150 cc.; reaction acid; color light golden yellow; specific gravity 1,021; albumin and sugar present; blood, bile, and casts absent.

Sheep 9 fell on the 15th day of feeding and could not rise. It was killed and autopsied on Nov. 22. Unusual changes were not noted.

The other animal, although badly affected with staggers, was able to stand, and after two feedings of green alfalfa it was turned out with two other sick animals (see Experiment 11).

Experiment 9.—Feeding of pampa grass to sheep that had recently recovered from staggers. Sept. 30, 1916. The feeding of pampa grass was begun with two sheep that had completely, and one that had partially recovered from staggers. These animals were part of a flock in which the disease had existed. They, with nine others, had been isolated in a small paddock about 2 weeks before our arrival and were to have furnished material for our investigation. Of these twelve animals, nine had recovered, two had become worse, and one had partially recovered. The partially recovered animal still showed extreme excitability and muscular trembling.

Oct. 5. One of the recovered sheep revealed unmistakable symptoms of staggers. The partially recovered animal had become much worse and fell on becoming excited. 2 days later the other hogget developed staggers. These animals all became worse and were either autopsied or used for other experiments.

When the three preceding experiments are reviewed it will be noted that of ten yearling sheep whose diet consisted of pampa grass all developed staggers. The controls fed on alfalfa hay remained well. Another striking feature is that both animals fed on fresh green pampa grass showed symptoms of the malady as rapidly as any of the others. The latter observation eliminates the theory of an infestation of the grass by a toxin-producing mold. The green grass we fed was free from mold.

It has been stated that old animals do not usually suffer from staggers unless they have been reared on low land and moved to the pampa. In instances of this kind, the losses may be severe. In the main, older animals that have been pastured on pampa cannot be said to suffer from the malady to any great extent. We are informed that the guanaco, a native member of the deer family, does not suffer from staggers. Indications on all upland paddocks visited showed that considerable coiron is eaten by animals. Often the green blades were kept well cropped. Knowing that more or less of the grass is eaten by older animals and that comparatively few cases of

staggers make their appearance in these flocks, we assumed that a tolerance to the toxic properties of the grass must have been developed. To test this point it was determined to feed the grass to older animals.

Experiment 10.—Nov. 14, 1916. In this experiment it was decided to feed adult animals on pampa grass. A 6 year old ewe with a 3 months old lamb, and two 2 year old wethers were fed upon pampa grass. The ewe could not support herself and the lamb on the diet and was discarded. The lamb showed symptoms of staggers within 8 days.

After 23 days of feeding on the grass, neither wether had developed staggers. Since it was necessary to return to New York, we requested the manager to continue feeding the grass and to telegraph us when the symptoms developed.

On Dec. 28, 45 days after the beginning of the feeding, he telegraphed us that both animals had staggers.

When young animals were fed on the grass, the average time for the first appearance of definite symptoms of the disease was 10 days. At the end of 23 days neither of the older animals revealed suspicious symptoms; in fact, 45 days of actual feeding were required to produce the disease. It seems that a considerable tolerance is developed in adult animals that have pastured on land on which the grass grows. The results of this experiment readily explain why these sheep do not suffer from the disorder to any great extent. In all probability the guanaco, through generations, has developed an immunity against the toxic substances of the grass.

Experiment 11.—In spontaneous outbreaks of the disorder the mortality is usually slight when compared with the incidence. In fact, in certain outbreaks all the animals recover if there is sufficient green fodder.

Sept. 16, 1916. Three yearling rams were brought into the settlement by the manager and placed in a small corral; these animals presented all the symptoms of staggers and would fall on becoming frightened (Fig. 10). They were of value for breeding purposes and the manager determined to treat them. Sept. 16 and 17. Each was given a pint of oatmeal gruel and permitted to eat rolled oats and both dry and green alfalfa. The bulk of the diet consisted of alfalfa hay. Within 1 week the improvement was marked; even when they were driven by dogs they did not fall. The symptoms rapidly disappeared and within 18 days all had recovered (Fig. 11).

In another instance, two animals, the lamb from Experiment 10 and a hogget from Experiment 8, together with a spontaneous case, were allowed to run in a small paddock where the finer grasses were plentiful. One of the animals could not be driven but had to be carried to the paddock; at the least excitement it

would fall and only rise with difficulty. Within 8 days all symptoms had disappeared and all three sheep had completely recovered.

Experiment 12.—Attempts were made to extract some substances from the grass that would cause the symptoms. Acosta was unable to state definitely whether the plant itself contained an alkaloid or whether a toxin was produced by the action of a mold. The former seemed more plausible as Patagonia is an exceedingly dry country. Moreover, the experiment in which we fed green grass which was not moldy seems to point to an alkaloid.

At first we attempted to extract a substance from the plant with alcohol, but on account of our small supply of that chemical we were compelled to discontinue this portion of the experiment.

We next attempted to extract some substance aqueously. This was done in the following manner: 3 kilos of grass were washed twice and then covered with water and allowed to boil for 2 hours. The liquid was then poured off and used to cover a second 3 kilos of washed grass. This was repeated a third time. The dark brown liquid thus obtained was evaporated to 1 liter. It was necessary to prepare a large quantity 3 or 4 days before the administration of the liquid was begun, to enable us to continue the supply.

Two yearling hoggets were chosen for the experiment. The fluid was administered directly into the rumen by means of a stomach tube. Each animal received 1 liter before feeding in the morning and another liter in the evening, so that each animal was receiving daily the infusion from 18 kilos of grass. The experiment was continued for a period of 33 days, but symptoms failed to develop in either of the sheep.

Guinea pigs, when injected intraperitoneally with heavy alcoholic or aqueous extracts of the plant, usually died within 15 minutes. Controls inoculated with similar preparations from non-toxic grasses died as promptly. It was impossible to attempt to purify the products and it seemed that these results may be accounted for by an excess of salts in the fluids.

A young hogget was injected intraperitoneally with a large dose of a heavy grass infusion. It developed peritonitis and died within 12 hours. Symptoms of staggers were not observed.

It seemed that animals affected with staggers or those that had recovered might react when a few drops of liquid thought to contain the toxic substance were placed in the eye. Both alcoholic and aqueous extracts were evaporated to dryness and the residue was taken up in distilled water. Normal, affected, and recovered animals received two drops of these solutions in each eye. All failed to react.

Probably only more refined and exhaustive chemical methods will be able to decide the nature of the substance which is responsible for the symptoms.

DISCUSSION.

There seems to be very little doubt that staggers in sheep in Patagonia is caused by ingestion of a coarse tuft grass. This plant is called pampa or coiron grass. Its botanical name is *Poa argentina* (Fig. 12). All young sheep (yearlings) when fed on this grass developed characteristic symptoms of the disease. Besides our experimental evidence, there exists a number of other facts which corroborate our findings. Certain of these can best be illustrated by a map of an estancia on which staggers is prevalent (Text-fig. 1).

The larger paddocks consist of broken pampa; *i.e.*, pampa intersected by valleys. In all of them except the Windmill Paddock, the water supply is natural. The windmill wells have been driven. In all the upland paddocks staggers exists. The pampa grass grows well. On this property there are three low paddocks (Nos. 1, 2, and X) where this particular grass does not grow. These paddocks are at least 200 or 300 feet lower in altitude than the pampa. No matter how sparse the herbage may become in them staggers never develops in any of the sheep ranged there. This company owns a piece of land six leagues to the eastward and only a few feet above sea level; on this tract the disease does not exist and the coiron grass is not found. When a flock of 2 year old ewes was moved from these low camps to the mixed pampa paddocks, staggers broke out.

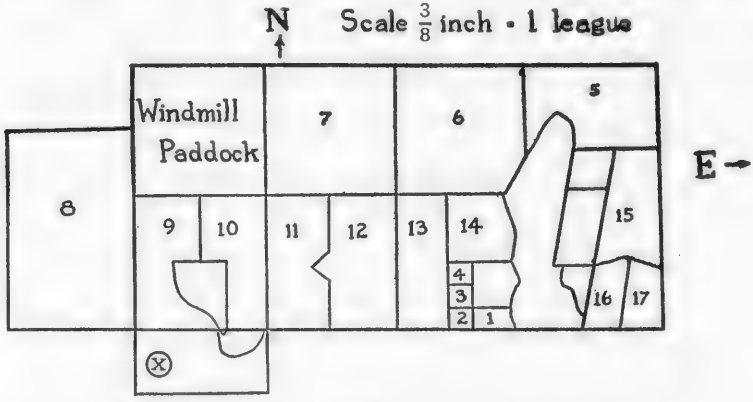
The question may be raised whether the symptoms may be caused by a lack of some vital substance in the diet; *i.e.*, whether staggers is an insufficiency disease. There are several facts which lead us to believe that the disorder is not of such a nature. When horses are brought on the pampa from other districts they often come down with staggers within 24 hours. We have seen sheep reveal symptoms after two feedings of pampa grass. In years of drought many animals die of starvation in the low camps where the pampa grass does not grow but the other food is the same. These sheep do not develop nervous manifestations.

Theiler, Green, and Viljoen[7] have shown that even after long periods

[7] Theiler, A., Green, H. H., and Viljoen, P. R., Contribution to the study of deficiency disease, with special reference to the Lamkziekte problem in South Africa, *Third and Fourth Reports, Union of South Africa, Dept. Agric.*, 1915.

horses, cattle, and sheep fed on a diet of ten parts of polished rice and one part of autoclaved hay failed to develop symptoms of an avitaminosis.

It would seem that the solution of the problem lies in the eradication of this particular grass (Fig. 13). When the fact is considered that this grass is abundant from the foot-hills of the Andes to the eastern boundary of the pampa, one gets an idea of the magnitude of the problem. During the summer months when the grass is seeding, the prevailing winds are from the west and it is not unusual for them to blow steadily for 3 or 4 days. At one time during our stay the



TEXT-FIG. 1. Map of an estancia on which staggers is prevalent.

wind reached a maximum velocity of 84 miles an hour. Even though this grass is eradicated from eastern portions of the land it would soon reappear. One land owner tried burning off a portion of land but this failed to kill the coiron. Unless another grass can be substituted, the eradication of this particular species might work considerable havoc. There is no doubt that old sheep that have become tolerant eat it without harmful effects. Probably during periods of drought many animals are dependent on it for certain nourishment. Without the substitution of some other bulky food the losses from

starvation might well equal the losses occurring from staggers in continued periods of drought.

Individual treatment is valueless on large estancias at the present time. Many of the valleys in this district are admirably suited to the raising of alfalfa after proper treatment of the soil. Generally two good cuttings may be obtained annually after a few years. In the future when the value of sheep has advanced it may pay to cultivate portions of the better valleys and either use the alfalfa as a green forage or store it as hay or ensilage and feed it in times of stress. It is recognized at this time that this procedure is impracticable, although it may become necessary in the future.

Sheep raisers have long recognized the advisability of not exciting flocks in which many cases of staggers exist. Usually when flocks in which the incidence is heavy are driven by men and dogs, the sheep, becoming violently excited, attempt to run and many of them fall down. Of those that fall many are unable to rise and ultimately die of starvation or are killed or mutilated by animals or birds. Quiet is essential when many are sick. On the other hand, it may be advisable to move sheep to another pasture early in the outbreak if this is possible.

Acosta recommends the use of nose bags when sheep are driven through lands on which the grass exists. When it is considered that two or three men with dogs usually drive bands of a thousand or more sheep, such a procedure seems impossible.

Staggers affects horses and cattle as well as sheep. Acosta was able to produce the disease experimentally in both species by feeding *Poa denudata*. Apparently *Poa denudata* and *Poa argentina* differ in but one or two minor characteristics. Opportunity for experiments along this line was not afforded to us.

CONCLUSIONS.

After observations and experimental work both in the field and laboratory, the following conclusions seem justified.

1. Staggers is a non-infectious disorder affecting horses, cattle, and sheep.

2. The disease is characterized by weakness, muscular twitching, irregular movements of the head, stiffness of the limbs, and transient

motor paralysis, accompanied with spastic spasms on excitement. There is also a derangement of vision and conjunctivitis.

3. The postmortem lesions are not characteristic.

4. We readily produced the disease by feeding susceptible sheep on a coarse tuft grass commonly known as coiron or pampa grass (*Poa argentina*).

5. The time required to produce definite symptoms by feeding the grass varied. Two animals developed typical staggers after two feedings; in another instance a period of 21 days of feeding was required. The average time for the production of unmistakable symptoms in our experiments was 10 days.

6. Many sheep recover from staggers spontaneously. A complete change of diet will usually effect a cure within 2 weeks.

7. Older animals that have pastured for long periods on lands where the grass grows become tolerant and are rarely affected with staggers.

8. The grass is toxic to sheep at all seasons of the year. We fed late winter and early spring grass and grass in flower, and produced staggers in every instance. The young green grass is as toxic as any edible portion of the plant.

EXPLANATION OF PLATES.

PLATE 62.

FIGS. 1 to 5. Spontaneous staggers.

FIG. 1. Animal about to fall on its side; the fore legs are rigidly extended and the digits spread far apart.

FIG. 2. The transient motor paralysis of the hind limbs occurring after violent exercise and excitement.

FIG. 3. Sheep about to fall. All four legs are extended rigidly. The momentum gained was sufficient to carry the body forward. The animal appears to plunge forward while falling.

FIG. 4. The gait of sheep with staggers; note the apparent rigidity of the hind limbs.

FIG. 5. The usual position of an animal down with staggers.

PLATE 63.

FIGS. 6, 7, and 8. Experimental staggers.

FIG. 6. Sheep 1, Experiment 6. Note the stiff carriage of the hind limbs and the depression of the hind quarters.

1

2

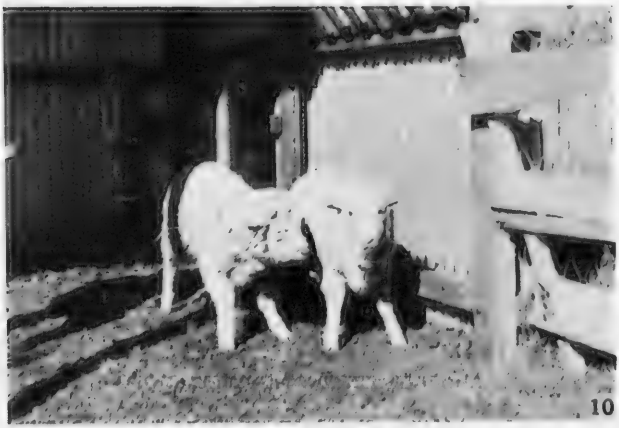
3

4

5

(Jones and Arnold: Staggers in sheep.)

(Jones and Arnold: Staggers in sheep.)



(Jones and Arnold: Staggers in sheep.)

(Jones and Arnold: Staggers in sheep.)

FIG. 7. Sheep 7 and 8, Experiment 7. Typical standing position of sheep affected with staggers.

FIG. 8. Sheep 8, Experiment 7. This plate was exposed $\frac{1}{2}$ second and shows the characteristic shaking of the head.

PLATE 64.

FIG. 9. The sheep fed on green pampa grass (*Poa argentina*) in Experiment 8.

FIG. 10. Young rams affected with staggers (Experiment 11).

FIG. 11. The same animals as in Fig. 10, completely recovered after feeding for 18 days on a mixed diet of alfalfa and rolled oats.

PLATE 65.

FIG. 12. Pampa grass (*Poa argentina*) in flower.

FIG. 13. The grass growing on the pampa close to the edge of a valley.

THE DIVERSION OF THE PANCREATIC JUICE FROM THE DUODENUM INTO THE STOMACH. ITS EFFECTS UPON THE LEVEL OF GASTRIC ACIDITY AND UPON THE PANCREAS.

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Recent work has shown the important part played by the alkaline duodenal contents, the pancreatic juice in particular, in the maintenance of a uniform level of gastric acidity. While the presence of bile and pancreatic juice in the stomach was noted a number of years ago by Boas, it remained for Boldyreff (1907–08, 1911) to demonstrate experimentally that a regurgitation of duodenal juices, after the ingestion of certain foodstuffs, is of such regular occurrence that the process may be regarded as an accompaniment of normal gastric digestion. Confirmatory evidence for the existence of the mechanism responsible for this regurgitation has since been offered by Carlson (1915, *b*) Hicks and Visher, Zaitseff, Spencer, Meyer, Rehfuss, and Hawk, Morse, and others.

It has been demonstrated, furthermore, that neutral, alkaline, or feebly acid fluids provoke a secretion of acid juice in order that the gastric acidity may be brought to the normal level. The experiments of Lönnqvist, for example, have shown that when weak soda solutions (0.25 to 0.5 per cent) are introduced into the stomach they act like water in causing a secretion of gastric juice. With stronger concentrations (1.0 to 1.5 per cent) the amount of secretion is materially increased. While it is not inconceivable that strong alkaline solutions, acting as an irritant to the duodenal mucosa, may lead, indirectly, to an outpouring of acid in the stomach, it is not likely that the weaker solutions act upon the gastric glands in this way. In fact Lönnqvist found that when solutions of soda were placed in the duodenum they led to a slightly diminished secretion of gastric juice.

There is but little evidence, however, to show how the level of gastric acidity is affected when alkaline solutions are introduced into the stomach, more or less continuously throughout the period of digestion. In view of the findings of Boldyreff (1911) which indicate that the gastric contents become more acid (hyperacidity) when there is an insufficient regurgitation of duodenal juices, one might expect to find a lowered level of acidity in the presence of an excessive duodenal regurgitation. When fats are fed in considerable amounts they bring about

a profuse influx of duodenal contents into the stomach, and at these times there is a decrease in the acidity level. But, as fat is only slightly affected by gastric juice, these experiments merely serve to demonstrate how the duodenum may aid the stomach in its struggle with an unusual burden.

While it would be difficult, experimentally, to increase or prolong the duodenal regurgitation without introducing fat or strongly acid solutions into the stomach, evidence bearing on this problem may be obtained by preparing animals in such a way that an unusual amount of bile or pancreatic juice enters the stomach throughout the period of digestion. In an earlier report (Grey) experiments were cited in which the total output of bile was made to flow through the stomach in its passage to the intestines. The present communication concerns certain additional experiments in which the pancreatic juice was diverted from the duodenum into the stomach.

The results of the first investigation appeared to indicate that the presence in the stomach throughout the course of digestion of the entire output of bile does not materially affect the level of gastric acidity. While no interpretation of these findings was attempted in the published report of the experiments, it was apparent, nevertheless, that they did not necessarily imply that bile is a feeble, neutralizing agent, but suggested, rather, that there had been a compensatory increase in the secretion of hydrochloric acid whereby an approximately normal level of acidity was maintained.

The analysis of the pure juice obtained from the smaller (Pawlow) stomachs did not reveal any appreciable changes in the hydrochloric acid content of the secretion secured subsequent to the introduction of bile. This observation was in accord with the findings of Carlson (1915, *b*) which point to a constant and uniform acidity of the digestion juice when it is secreted at a fairly high rate. A uniformly lower acidity is found under other circumstances; *i.e.*, in the empty stomach and in the continuous or hunger secretion, when the secretion rate is low.

The protocols of these experiments, however, indicate that there was a definite increase in the quantity of the digestive juice secreted after the bile had been diverted into the stomach. This conclusion has been based upon a comparison of the average of between twenty-five and thirty measurements made previous to the anastomosis with a similar average of a like number of measurements carried out subsequent to the operation. Inasmuch as there is some variation both in the quantity and in the acidity of the specimens secured from day to day under approximately constant conditions, it is not feasible to compare the data from any two single observations. The figures taken from the protocol of Dog 2 and published in Table I of the report under discussion were chosen because they furnished an accurate picture of the average acidity values, before and after the anastomoses. They indicate, however, differences only of about 7½ per cent in the two outputs. In this respect the table was misleading since a comparison of the average amounts of juice secreted before and after the anastomoses revealed a considerably greater difference than this.

It was obviously impossible to make quantitative studies of the hydrochloric acid output in the animals in which samples of the gastric contents were analyzed—apomorphine experiments. But since no material change in the acidity level was noted after the introduction of the bile, it seems justifiable to infer that a compensatory increase in the gastric secretion had ensued to counterbalance the unusual quantity of alkaline fluid in the stomach.

Analyses of the secretions of the upper intestinal tract have shown that a number of them are capable of neutralizing more or less acid in the gastric juice; *i.e.*, saliva, gastric mucus, pyloric juice, the secretion of Brunner's glands, succus entericus, bile, and the pancreatic juice. Of these the pancreatic juice is by far the most important, since the alkalinity of this secretion is half again as great as that of all the other secretions combined (Boldyreff, 1914–15).

Because of the marked alkalinity of the pancreatic juice and in view of the results obtained from the bile experiments, it seemed important to study the effects on the level of gastric acidity of an increased supply of pancreatic juice in the stomach. In the experiments to be cited the larger pancreatic duct of each animal was transplanted into the wall of the stomach and the lesser duct ligated and divided.

This procedure afforded an opportunity to make certain additional observations. It has been the view of some workers that the hydrochloric acid in the duodenum is responsible for certain of the pathological changes which are found in the pancreas. Hlava, for example, suggested that by antiperistalsis hyperacid gastric juice may be forced from the intestine into the pancreatic duct, thus causing hemorrhagic pancreatitis. If this postulation is valid in any sense, then an experimental procedure such as that mentioned should afford a favorable opportunity for the gastric juice to reach the gland and set up acute inflammatory or chronic sclerotic changes.

Flexner injected hydrochloric acid solutions ranging in strength from 0.5 to 2 per cent into the pancreas and through its duct and subsequently observed profound changes in the organ corresponding in degree to the amount of acid used. The pathological picture was that of hemorrhagic pancreatitis. However, though these studies demonstrated that hydrochloric acid, as well as certain other agents, is capable of producing an acute inflammatory degeneration, Flexner did not offer his results as evidence confirming the hypothesis of Hlava.

Dr. Halsted's instructive case of stone in the ampulla of Vater and Opie's experiments have shown the great significance of one of the duodenal juices—bile—in pancreatic diseases. But the manner of entry of the bile into the pancreatic duct in these instances is essentially different from that suggested by Hlava. In Opie's opinion there is no satisfactory proof to indicate that the hydrochloric acid is instrumental in bringing about such a disease.

Methods.

The present report is based upon the results obtained from experiments carried out on seven dogs. A number of additional animals were used in the early part of the work, but they were employed solely in solving the surgical aspects of the problem.

Inasmuch as the principal purpose of the investigation was to follow the changes in the acidity level of the stomach, it seemed best to analyze specimens of the test meals instead of studying the pure gastric juice as was done in some of the bile experiments referred to above. For this purpose the gastric fistula as devised by Janeway proved to be satisfactory. All the animals were anesthetized with ether.

A small three-sided piece of stomach wall was turned down toward the greater curvature. This constituted a rectangular flap with its blood supply intact. By sewing the lateral margins together and closing the opening in the gastric wall a small tube was formed which was lined throughout by mucous membrane. The distal end was then sewed into the abdominal wall, care being taken to anchor it to the peritoneum, fascia, and skin. The mucosa and skin, of course, were sutured together. In experimenting with the reconstruction a very important feature was noted. During the first weeks subsequent to the operation there is a marked retraction of the new tube toward the peritoneal cavity, with a definite contraction of the external opening. The effects of these changes, however, may be greatly minimized if care is exercised during the operative procedure to anchor the tube in the abdominal parietes so that it protrudes for several centimeters above the level of the skin. This may be done without embarrassing the blood supply. It leaves a wide cuff of gastric mucosa exposed on the surface. When the skin margins are loosely attached to the latter, a considerable contraction of the opening may

take place without in any way stenosing the canal. Fistulas constructed on this plan will remain patent for many months. Frequent catheterization with only the most gentle manipulations serves to keep the tract clean.

Inspection of one of these fistulas 3 or 4 months subsequent to its construction shows a lead-pencil-like tube, 4 or 5 cm. in length, extending through the peritoneal cavity from the abdominal wall to the stomach. This arrangement probably interferes but little with the normal gastric movements. After the heartiest meal and even during emesis there is no loss of stomach contents from the canal.

While the experiments are still in progress concerning the transplantation of the pancreatic duct, devoid of duodenum, into the stomach wall, no satisfactory results of this nature were secured during the course of the work reported here. However, it was found possible to resect a very short cylinder of duodenum, approximately 2 cm. in length, containing the orifice of the major pancreatic duct, and, after closing one end, to transplant the opposite opening into the posterior wall of the stomach. Since this segment of small bowel devoid of its blood supply may atrophy and lead, as one of the earliest specimens demonstrated, to a stenosis of the duct, it was found necessary always to preserve the vessels of the transplanted duodenum. When care is exercised this may be accomplished without great difficulty. An end to end anastomosis then established the continuity of the duodenum. The lesser pancreatic duct which opens into the duodenum together with the common bile duct was now identified by dissection, doubly ligated, and divided.

While the transplantation of the pancreatic duct leads to a displacement of the adjoining parts of the pancreas, the new arrangement does not cause undue tension on the vessels which supply this organ. The operation is readily carried out through a high right rectus or midline incision.

A standard mixture of 70 gm. of ground, raw, lean beef and 75 cc. of tap water was used as a test meal. Samples were withdrawn for analysis 2 and 3 hours after the ingestion of the meal. These periods were chosen for two reasons. In the first place, it was found that at shorter intervals following the ingestion of the test meals the food had not undergone sufficient digestion; and after longer intervals

it was frequently very difficult to secure specimens for analysis, the ingesta apparently having left the stomach in large part. In the second place, it has been shown (Carlson, 1915, *a*) that the quantity of gastric juice yielded by a dog's accessory stomach (Pawlow) during the first 2 hours on a moderate meal of meat is about one-half that secreted for the entire digestive period. And from McClendon's experiments on man it appears that the acidity of the adult stomach rises during the first $1\frac{1}{2}$ to 3 hours subsequent to a meal, after which it remains stationary until the food has nearly all left the stomach.

Care was taken to conduct the observations in the forenoon 18 hours after the last meal. The dogs received the same diet each day. Water was withdrawn after the test meals had been given. But throughout the balance of the day the animals were permitted to take as much water as they desired. The amount of exercise, the quantity of food, and the environmental conditions remained constant throughout the weeks of study.

Several dozen test meals were given in each case. The average results obtained under these conditions were taken to represent fairly accurately the normal acidity of the chyme. In withdrawing samples for analyses care was exercised to discard the contents of the fistula itself. The end of a soft rubber catheter gently introduced a few centimeters into the stomach cavity was connected with a stoppered flask. Attached to one arm of the latter was a large syringe. The suction created by withdrawing the plunger usually resulted in a discharge of sufficient juice for study.

For the quantitative estimation of trypsin in the gastric contents Spencer's modification of the Ehrenreich test was used.

The acidity determinations were made by titrating 1 cc. of the sample against $\frac{N}{40}$ sodium hydroxide, using dimethylaminoazobenzene and phenolphthalein as indicators. The values have been expressed as the number of cubic centimeters of 0.1 N sodium hydroxide necessary to neutralize 100 cc. of gastric contents.

RESULTS.

Early in the course of the work it was noted that when test meals were given to the same animal on different days, under conditions

as similar as possible, samples of the gastric contents showed considerable variation in the hydrochloric acid content. Corresponding observations had been made during the course of the bile experiments discussed above. It was necessary, accordingly, to repeat the analyses a great number of times and average the results in order to secure an accurate picture of the acidity level at the periods of digestion chosen; *i.e.*, 2 and 3 hours after the ingestion of the meal. This mean was established both before and after the transplantation of the duct for each animal.

TABLE I.

Dog No.	Before transplantation of the duct.		After transplantation of the duct.		Interval between the fistula operation and the transplantation.	Interval between the transplantation and the first analysis.
	2 hrs.	3 hrs.	2 hrs.	3 hrs.		
					days	*days*
1*	104†	93	113	67	30	33
2*	107	100	122	74	32	22
3*	108	122	125	98	45	28
4*	103	115	112	83	47	29
5*	91	79	85	57	54	15
6*	96	82	91	67	55	20
7‡	112	120	108	73	107	74

* Test meal: 70 gm. of meat plus 75 cc. of water.

† Acidity values are expressed as the number of cubic centimeters of 0.1 N sodium hydroxide necessary to neutralize 100 cc. of gastric contents.

‡ Test meal: 100 gm. of meat plus 70 cc. of water.

In Table I the acidity values for each dog represent the average of the figures obtained in from ten to thirty separate analyses. The necessity for this mean has been emphasized by Foster and Lambert who believe that limitations of variability must be computed for each animal experimented upon, and that the only idea of limitation of function that we can gain must depend upon an average computed from the data for a number of animals. In the paper of Long and Hull the tables indicate a similar variation of the acid content from day to day.

In order to ascertain whether any relation might exist between the level of gastric acidity and the interval of time elapsing from the date

of the operative procedure to that of the analyses, the time factor was changed throughout the experiments. Though the animals were studied over varying periods, from 100 (Dog 5) to 228 days (Dog 7), no such relation was discernible.

From a study of the figures in the table it is evident that in three animals (Dogs 5, 6, and 7) the acidity level of the gastric contents was lowered by the introduction of pancreatic juice into the stomach, both at the 2 hour and at the 3 hour period of digestion. In Dog 7 the reduction was especially marked during the second period. On the other hand, the analyses in four of the animals (Nos. 1, 2, 3, and 4) showed a slight increase of acid in the 2 hour specimen, but a decrease in the specimens secured at the 3 hour period. While four of the seven dogs were thus found to have a higher level of gastric acidity subsequent to the transplantation of the pancreatic duct at the 2 hour period, it is noteworthy that in all the animals the acidity level fell at the 3 hour period. Moreover, in each of the latter, following the transplantation of the duct, the percentage of hydrochloric acid in the stomach contents was less at the 3 hour period than it was previous to the operation at the 2 hour period.

A further analysis of the table shows that in three dogs (Nos. 3, 4, and 7) before the pancreas had been disturbed, specimens of test meal contained more acid 3 hours after the ingestion of the food than an hour earlier than this; whereas the relationship was reversed in the remaining animals. These findings probably have their explanation in the fact that the dogs varied in size. In the smaller animals the process of gastric digestion was more prolonged. Subsequent to the operations, however, the specimen secured in the second period of digestion, in every case, contained less acid than that withdrawn during the first period. The constancy of this finding we may fairly attribute to the altered chemical conditions in the stomach induced by the pancreatic juice.

The experiments as a whole thus appear to indicate that the presence of a large quantity of pancreatic juice in the stomach throughout the period of digestion leads to a moderate reduction of the acidity level in the later stages of digestion. Earlier in the process there is no constant alteration of the acid content in either direction. The surprising feature of these results would seem to be the maintenance

of a relatively high acidity level in the presence of the large amount of pancreatic juice.

In view of the part played by the pancreatic juice in the self-regulating mechanism described by Boldyreff one might have anticipated, under these circumstances, a material reduction in the acidity level. The absence of such a result, however, is really in keeping with Boldyreff's hypothesis.

One of the controlling factors in the mechanism is the mucosa of the duodenum. Hyperacid stomach contents reaching this part of the digestive tract irritate the mucous membrane, and the latter, in turn, serves to bring about a reduction of the acidity level of the stomach through a regurgitation of alkaline duodenal juices. This type of self regulation, however, ensues only when the hydrochloric acid content of the chyme is above normal. When alkaline materials have lowered the acidity level a different process is called into action.

Experiments have shown that following the introduction of alkaline solutions into the stomach the gastric glands respond with a discharge of juice in an endeavor, it would appear, to restore the gastric acidity to the normal level. The presence of a considerable quantity of pancreatic juice in the stomach, of course, leads to a similar disturbance of the acidity level. Accordingly under these circumstances one would also expect to find an increase in the gastric secretion. Now it has been shown (Table I) that a more or less continuous influx of pancreatic juice into the stomach throughout the period of gastric digestion only depresses the acidity level in the later stages of digestion. This we can explain only by assuming that in the experiments mentioned there was an augmented secretion of hydrochloric acid throughout the digestive cycle corresponding to the continuous influx of alkaline fluid from the pancreas. These quantitative changes in the outflow of the juice were observed in certain of the bile experiments, referred to above.

In the work hitherto reported the activity of the stomach, as far as it concerns this phase of the self-regulating mechanism, has only been followed by the introduction into the stomach of a given quantity of some alkaline solution. These experiments, however, afford us an opportunity to watch the reactions of the stomach to alkaline material repeatedly administered throughout the digestive cycle.

The results obtained serve to draw attention to the remarkable capacity of the stomach to maintain a relatively high acidity of its contents. In the past perhaps undue emphasis has been laid on the occurrence of duodenal regurgitation. We shall probably be more exact in our conception of the mechanism which controls the level of gastric acidity when we constantly bear in mind that it, in turn, depends upon two more or less distinct, subsidiary mechanisms. One of these concerns the neutralization of hyperacid chyme through duodenal regurgitation. The other, a gastric process, provides for an outpouring of gastric juice when the stomach contents are below the normal acidity.

These findings have more or less clinical significance since it is not at all certain, at present, how much of the favorable results obtained from the medical treatment of gastric ulcer is due to a lowering of the acidity level. Carlson (1917) suggests that the relief from pain following the administration of alkalis or following gastroenterostomy may be due to other factors than the effect on the gastric juice. These factors may also explain, in part, the excellent results observed after the Finney pyloroplasty since this operation affords ideal conditions for the entrance of duodenal juices into the stomach.

The possibility, of course, must be borne in mind that subsequent to the transplantation of the duct into the stomach wall, the pancreas may have ceased discharging juice. Against this assumption, however, there is more or less conclusive evidence. In the first place, the results from the analyses indicate that there was a slight depression of the acidity level at the 3 hour period of digestion after transplantation of the duct, a finding to which there was no exception among the seven animals used. This pointed to an alteration in the chemistry of the stomach. In the second place, histological study of the parts of the pancreas adjacent to the large duct disclosed no discernible pathological changes such as would be expected were the duct occluded. In Hess' experience ligation of the ducts of the pancreas leads to a sclerosis of the entire gland. The amount of sclerosis is proportional to the size of the duct which has been ligated, together with the number of its communications. What is more, special stains demonstrated that the cells in this neighborhood were charged with secretion granules and, accordingly, that they were in an active

state. In the third place, the results obtained from the trypsin determinations pointed toward the presence of an unusual quantity of pancreatic juice in the stomach subsequent to the transference of the duct from the duodenum to the stomach. And, finally, dissection of the specimens removed at necropsy showed in each instance a patent duct opening into the small duodenal transplant, with a spacious communication between the latter and the stomach. In one animal the postmortem examination was made about 120 days subsequent to the operation on the pancreas.

The most convincing evidence that pancreatic juice is regularly discharged into the stomach after this plastic operation is afforded by the results which have been obtained in some more recent experiments concerning duodenal extirpation. While this work is still incomplete it has nevertheless shown that in duodenectomized animals where the major pancreatic duct has been transplanted into the gastric wall the pancreas remains histologically normal at the end of the 5th week. For reasons which will be discussed in a subsequent communication these animals have succumbed at about the end of that period. The fact, however, that no changes of an atrophic nature were ever discovered in the pancreas at postmortem examination, together with the invariable finding of a definite gastritis, furnish reliable evidence that the pancreas continued to discharge its external secretion into the digestive tract through its new communication with the stomach.

Making use of a modification of Ehrenreich's test, Spencer, Meyer, Rehfuss, and Hawk studied the trypsin values of the gastric contents in man under varying conditions. The results of their experiments serve to substantiate the regurgitation hypothesis of Boldyreff, and indicate that tryptic digestion frequently proceeds in the normal stomach. In view of this work an attempt was made to follow the trypsin values of the stomach contents from two dogs (Nos. 3 and 7) both previous and subsequent to the operation on the pancreas. The test was repeated a considerable number of times with each animal.

Practically no digestion was ever noted in the tests carried out on normal animals. Following the transplantation of the major pancreatic duct into the stomach, however, there was a definite digestion of casein. A certain variation in the tryptic activity of the gastric

contents was appreciable from day to day, but this did not serve to obscure the difference referred to.

The new conditions established by the transplantation of the duct probably led to definite changes in the rate and character of digestion, both in the stomach and in the duodenum. Many believe that gastric juice is peculiarly destructive to the pancreatic ferment by virtue of the action of hydrochloric acid on the enzyme. As Long and Hull have shown, however, this may not actually be the case. Some tryptic digestion may occur within the stomach provided the free acid remains sufficiently low through protein combinations. Interesting as this aspect of the problem is, no further observations concerning digestion were carried out.

The animals were kept under observation for varying periods. Dog 7 was killed 121 days subsequent to the transplantation of the pancreatic duct. At that time it was in perfect health, having weaned a litter of well nourished pups a few days previously. In the series of seven dogs there was one death (Dog 1). This animal was active and appeared to be in good condition up to a few days before it died. At autopsy no satisfactory cause of death was discovered. There were many intestinal worms, but, as most of the laboratory animals harbor parasites of this nature, the findings may have no practical significance. Of the remaining five animals three were moderately well nourished and two rather undernourished when they were killed. All of them, however, ate heartily of their food.

Postmortem examination revealed an interesting condition at the site of the transplantation. In each animal there was a tiny pouch of duodenum attached to the stomach. The line of demarcation between the two was clearly evident, and as far as the macroscopic features were concerned, there was little to differentiate the transplant from the neighboring duodenum. It retained some, if not all, of its original blood supply.

The major pancreatic duct was readily found opening into the base of the pouch. Inspection of the wall showed it to be soft and of normal caliber, and on instrumentation the duct admitted a relatively large probe. There were no signs of atresia of the duct in any of the seven specimens examined. A search was made in each case for any possible accessory communications between the gland and the duo-

denum. We know from the studies of Hess on the canine pancreas that there may be three and occasionally four ducts opening into the duodenum. The main duct which arises from the union of two large branches drains the principal canal system of the pancreas. The accessory duct lies more oralward and opens into the intestinal lumen in association with the ductus choledochus. Between the two lies a smaller communicating channel. A fourth duct may connect the pars descendens with the duodenum.

In none of the specimens was the accessory duct found. But the search was not complete in any case since it was necessary to preserve the structures for histological study. In two of the seven animals a very small communication was found opening into the duodenum just proximal to the site of the end to end anastomosis. If a third duct of this type had been present in the other animals it seemed probable at the time of the necropsies that it had been destroyed in the course of the transplantations and the anastomoses.

The observations which have since been made on the duodenectomized animals, however, indicate clearly that some accessory communication between the pancreas and the intestine must have existed in each dog of the series, since the discharge into the stomach of the total output of pancreatic juice leads to profound changes in nutrition and to the development of a definite gastritis, and evidences of these processes were never discovered in any of the animals under discussion.

In gross the pancreas seemed normal in every way except that it gave the impression of being somewhat smaller than usual. The mucosa of the duodenal transplant, of the stomach, and of the intestine throughout its length showed no signs of ulceration or sclerotic changes. There was no injection even in the vicinity of the pancreatic duct. Lönnqvist has reported a temporary catarrhal condition of the gastric mucous membrane following a profuse reflux of bile and pancreatic juice into the stomach. There was nothing, however, either in the anatomical findings or in the characteristics of the samples of test meal removed to suggest this condition in any of these animals. But the experiences with duodenectomized animals have since demonstrated that pathological changes make their

appearance in the stomach as soon as the total quantity of the pancreatic secretion is diverted into the viscus.

Histological study of the pancreas disclosed no inflammatory or degenerative changes. It should be emphasized, nevertheless, that sections were prepared only from the central portions of the gland. Had the tissue adjoining the lesser duct been studied it is possible that the examination might have revealed some atrophic changes. In four of the specimens sections were prepared with the acid fuchsin-methylene green stain of Bensley. With this technique the cells were seen to be filled with secretion granules. The preparations examined were considered to represent normal tissue.[1]

The urine of one animal was followed for a number of weeks subsequent to the second operation (transplantation of the pancreatic duct). While some reducing substances were occasionally encountered the findings at no time suggested the presence of sugar.

SUMMARY AND CONCLUSIONS.

The mechanism described for maintaining the optimum level of gastric acidity is designated by Boldyreff as the "self regulation of the acidity of the contents of the stomach." In support of Boldyreff's hypothesis is the evidence obtained from many experiments carried out both on man and on animals, in which solutions of alkali and acid have been placed in the stomach. The introduction of acid fluid has led to a regurgitation of alkaline duodenal contents, whereas the introduction of alkaline solutions has called forth a secretion of acid gastric juice.

The experiments reported in this paper were carried out for the purpose of ascertaining how the stomach would react, in as far as the secretion of hydrochloric acid is concerned, to a more or less continuous influx of relatively strong alkaline fluid, prolonged throughout the cycle of digestion. Numerous studies have shown that any serious interference with the process of regurgitation leads to a rise in the acidity level of the stomach; *i.e.*, to a state of hyperacidity. There is but little evidence, however, to indicate whether the acidity level will be depressed temporarily or permanently (hypoacidity)

[1] Dr. E. Goetsch examined these preparations.

when alkaline material, in considerable amounts, continues to enter the stomach.

The influx of alkaline fluid was provided for by transplanting the larger pancreatic duct into the wall of the stomach after ligating and dividing the lesser duct. Specimens of test meal for analysis were withdrawn through gastric fistulas made after the method of Janeway.

Animals prepared in this manner served also to furnish additional information regarding the possible relation of the hydrochloric acid of the gastric juice to certain acute inflammatory and chronic sclerotic changes in the pancreas.

From the results of these experiments it appears that the presence of a considerable amount of pancreatic juice in the stomach throughout the period of digestion leads only to a moderate decrease in the acidity level of the ingesta in the later stages of digestion. Earlier in the process there is no constant alteration of the acidity level in either direction. The findings then serve not only to corroborate the views of Boldyreff, but also to demonstrate the remarkable compensatory activity of the gastric glands under conditions which entail an unusual quantity of alkali in the stomach.

In addition the work has shown that when the larger pancreatic duct is properly transplanted into the wall of the stomach, it may remain patent for months. In animals in which this operative procedure has been carried out, the pancreas has been found to undergo no inflammatory or other degenerative changes. This finding is regarded as evidence against the postulation of Hlava that gastric juice is probably responsible for the occurrence of certain cases of acute hemorrhagic pancreatitis.

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THE ABIOTIC ACTION OF ULTRA-VIOLET LIGHT.

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PLATE 66.

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INTRODUCTION.

The following data as to the bactericidal action of ultra-violet light on typhoid bacilli were obtained by a method different from that heretofore used and the results give additional evidence toward settling certain disputed phases of the subject.

In 1910[1] and again in 1912[2] Victor Henri and his associates published articles on this subject in which they give evidence to show that the bactericidal effect of ultra-violet light first becomes appreciable at 3000 Ångström units, and increases progressively from there downward toward 2150 Ångström units. The amount of this progressive increase they did not make clear. The evidence tended to show that there was no maximum in this region. The organisms were exposed directly to the ultra-violet light source, so arranged as to limit to a certain extent its spectral range. This limitation was effected by choosing three sources of ultra-violet light such that their intensities in various regions were quite different, and to emphasize further this difference the spectral distribution of their radiation was cut down by the interposition of one of six light-screens. It is difficult from the tables of data to follow them to their conclusions. The upper limit for the action of ultra-violet light they fixed by screening out the lower wave-lengths with glass and determining the amount of screening necessary to prevent the effect.

Browning and Russ[3] state that they prepared plates by making a thin spread of bacteria on glass-supported films of nutrient material. These were exposed in

[1] Cernovodeanu, P., and Henri, V., *Compt. rend. Acad.*, 1910, cl, 52, 549.

[2] Henri and Henri, *Compt. rend. Acad.*, 1912, clv, 315.

[3] Browning and Russ read a paper on this subject recently before the Royal Society which has not yet appeared in print. Their reading is abstracted, however, in the *Brit. Med. J.*, 1917, i, 656.

As this proof goes to print the paper of Browning and Russ has appeared (Browning, C. H., and Russ, S., *Proc. Roy. Soc. London, Series B*, 1917, xc,

the manner of a photographic plate at the back of a quartz spectrograph. The sterilizing action of the light gave a photograph of the active portion of the spectrum. They fixed 2960 Ångström units as the upper limit of effective light.

Lymann[4] has shown that the abiotic effect of ultra-violet light increases rapidly below 2000 Ångström units to become almost instantaneous at 1750. The lower limit of sensitivity has not been fixed. I have shown in a previous paper[5] that typhoid bacilli are slightly sensitive in the x-ray region (1 Ångström unit). There may, however, be one or more minima between 1 and 1750 Ångström units.

Method.

My experiments differ from previous ones in that water is used as a medium for the organisms. Experiments with the x-rays and with chemicals seem to show that nutrient media often protect the organisms from the action of abiotic agents. My experiments further differ from those of Henri and his associates in that the light incident on the organisms is confined in each case by the use of a quartz spectrograph to a very narrow region of the spectrum.

In the following experiments I have used a fresh water suspension of typhoid bacilli. A uniform quantity of the suspension was taken up on a platinum loop and used to fill a quartz capillary by capillary action. The quartz capillary was then fixed in an appropriate graduated rack in the focal plane of a quartz spectrograph and so

33). They found the cessation of abiotic action to be sharply at 2960 Ångström units for *Staphylococcus aureus*, and slightly higher, at 3000 Ångström units, for *B. typhosus*. An excellent plate for *Staphylococcus aureus* accompanying the article would indicate that the fall in abiotic action becomes perceptible at 2900 Ångström units and comes to zero at 2960 Ångström units. My quantitative data, graphically illustrated in Text-fig. 1, show that this fall to 2960 Ångström units is not so sharp as their plate would lead one to suppose. The probability that long exposures to sunlight have a killing effect would indicate that the curve of the figure approaches the horizontal axis asymptotically, that the curve is concave to the right as shown, rather than convex to the right, as an abrupt fall to zero would imply. My actual numerical data further imply this concavity. Browning and Russ required much longer exposures to kill the organisms than I did. My spectrograph is the same as theirs and my iron spark is probably not brighter than the tungsten arc which they used. The differences in exposure required by us would therefore seem to offer interesting corroborative evidence as to the protective effect of a nutrient medium for bacteria.

[4] Lymann, T., Spectroscopy of the extreme ultra-violet, London, 1914, 103.

[5] Newcomer, H. S., *J. Exp. Med.*, 1917, xxvi, 657.

exposed to a narrow region of the spectrum. After exposure the contents of the capillary were washed out and rinsed by suction in liquid agar and plated, the plates incubated, and counts made. The quartz spectrograph was a Hilger size C, giving a spectrum from 2100 to 8000 Ångström units 19 cm. long. The focal plane of the spectrograph formed an acute angle of about 30° with the light path. For most of the work the condensed iron spark was used as an ultra-violet source. It was placed 10 cm. from the entrance slit of the spectrograph. It was operated by a 10 kilowatt closed circuit transformer using 110 volts alternating current on the primary. The secondary voltage was about 10,000. The condensers had a capacity of 0.05 microfarad. The entrance slit of the spectrograph was opened to 1.5 mm., a width such that the individual lines of the iron spark were spread out into bands of the same width in projection as the quartz capillary. This produced an overlapping of the lines, giving the effect of a continuous spectrum. As a result any setting involved an exposure to a wave-length interval 15 to 40 Ångström units long. Fig. 1 reproduces a photograph of the iron spark spectrum, the upper band being the spectrum with a narrow slit, and the lower, the spectrum as used with a wide slit. The figure gives some information as to the relative intensity of light at different wave-lengths.

My data give the approximate wave-length range of the light falling on the quartz capillary for each setting. These wave-lengths were determined by measurements with a wave-length scale on photographs of the spark spectrum, the rack holding the capillaries being graduated to correspond with the wave-length scale. No attempt is made to give wave-lengths closer than to within 5 Ångström units. The data are computed on a basis of controls having counts of 100 colonies per sq. cm. The actual controls varied from 10 to 800 per sq. cm., usually about 150. When the controls gave more than 1,000 per sq. cm. the suspension contained so many organisms that some of them were protected from the light by being in the shadow of others.

OBSERVATIONS.

Table I gives the data for 10 and 5 minute exposures to the iron spark.

TABLE I.

Exposures to the Iron Spark of 10 and 5 Minutes' Duration.

Wave-length.	10 min. exposures.	5 min. exposures.	Wave-length.	10 min. exposures.	5 min. exposures.
Å. u.			Å. u.		
1990–2005	41*	73	2530–2560		0
1995–2010	50	62	2540–2570	0	3
2195–2215	8		2550–2580	0	
2205–2225	11		2560–2590	0, 0	½
2235–2255	25		2580–2610	0	0
2240–2260	28	50	2600–2630	0	¼
2250–2270	65		2610–2640	0	0
2270–2290	30		2645–2675	1	9
2280–2300	26		2650–2680	½	1
2290–2310	2,22	51	2655–2685	1½, 1½	3½
2325–2350	2½	4	2680–2715	0, ½	3, 3
2330–2355	1	14	2700–2735	0	1
2335–2360	2½	3	2760–2795	0	
2350–2375	3½	11	2780–2815	0	9,10
2365–2390	⅕	1½	2785–2825	1	
2385–2410	0, ½	0, 1½, 3	2790–2830	2	5
2405–2430	1/10	1¼	2795–2835	1½	4½
2435–2460	0	2	2845–2885	7	
2455–2480	1	2½	2850–2890		16, 17, 20
2485–2510	0, 0	0, 1, 1	2855–2895	3, 2½	5, 7½, 7½
2495–2525	0	1, 1, 2	2875–2915	7	
2500–2530	0	0	2945–2985	40, 45, 50	
2525–2555	0	¼			

* In the tables the data represent typhoid organisms per hundred remaining alive after the given exposure.

It is to be noted that the abiotic effect is low from 2100 to 2300. This corresponds to a portion of the iron spark spectrum of relatively weak intensity. In order to determine the nature of the effect in this region exposures were made to the copper and zinc spark, sources with lines of about equal intensity in this and higher regions of the spectrum. Table II gives the results of these exposures and shows that the effect is about the same in this region as elsewhere.

TABLE II.

Exposures to the Copper and Zinc Sparks.

Copper spark.		Zinc spark.		
Wave-length.	10 min. exposures.	Wave-length.	10 min. exposures.	5 min. exposures.
Å. u.		Å. u.		
2130–2140	57, 22	1990–2105	1	14
2180–2195	30, 23	2130–2145	5	21
2205–2225	30	2190–2210	37	
2235–2250	32	2250–2270	37	61
2265–2290	47	2490–2530*		1
2295–2320	79, 81	2545–2575*		0
2360–2385	24	2800–2840*	2	1½
2590–2620	47, 63			
2750–2785	49			

* Very strong lines.

The relative intensity of the light in the various portions of the spectrum could only be measured photographically, and comparisons were further made difficult by the fact that the plate gradually decreased in sensitivity in the far ultra-violet. Between 2100 and 2800 the exposures of Table I showing a slight relative decrease in abiotic effect correspond well with weak regions in the spectrum. Above 2800 the abiotic activity of the light begins gradually to fall off. This can be seen well in Table I and in Table III for 2 minute exposures.

TABLE III.

Exposures to the Iron Spark of 2 Minutes' Duration.

Wave-length.	2 min. exposure.	Wave-length.	2 min. exposure.
Å. u.		Å. u.	
2365–2390	13, 12, 13	2580–2610	1, 0, 1, 0
2385–2410	16, 20	2680–2715	22, 20, 21
2435–2460	13, 12½, 12	2850–2890	45, 52, 45
2530–2560	4, 2, 2, 1½, 1		

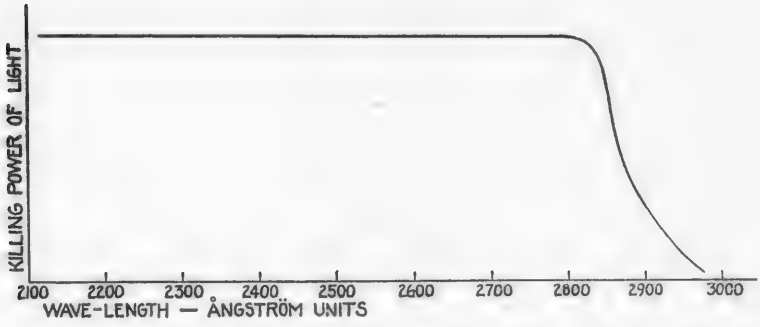
From Table III the decrease would appear to begin at 2700, but at this point the iron spark spectrum is rather weak. At 2750 the spectrum is very strong and here exposures corresponded with those made at 2600, also a relatively strong place in the spectrum. The

iron spark spectrum is slightly weaker below 2500 than above that point. Below 2320 it is very weak. Above 2780 it weakens perceptibly. About 2870 corresponds to a region of the spectrum equal in intensity to the 2700 region. At 2870 the tables show a decrease in abiotic activity. This decrease when corrected for the weakness of the spectrum amounts to a fall of one-half. The last appreciable abiotic effect occurs somewhere between the moderately strong 2950 and 2990 lines. In this region the intensity is about the same as at 2700 and 2870. At 2945 to 2985 exposures for 10 minutes averaged 55 per cent killed, for 20 minutes 81 per cent killed. An average of eight exposures at 2990 to 3030 for 20 minutes gave no mortality. These exposures at 2945 to 2985 and 2990 to 3030 were controlled for evaporation and for scattered light. Longer exposures were impracticable because of the increasing influence of both these factors, particularly the former. Complete evaporation of the contents of the capillary occurring in about 40 minutes will completely or almost completely kill the typhoid organisms.

In order to limit further the upper end of the abiotic spectrum exposures were made directly before the iron spark but behind a piece of optical glass 3 mm. thick. The distance from the spark was 4 cm., the increase in energy due to lessened distances alone amounting to 256 times. Besides this, the whole area of the spark was effective rather than a portion corresponding to the slit area. This introduced another factor so that there was an increase of about one thousandfold. The glass transmitted no light below 3100; above 3300 it was almost transparent. These exposures of 5 and 10 minutes' length had no effect on the typhoid organism. The terrestrial sun's spectrum ends somewhere near 2970. Exposures to sunlight of 20 minutes' duration produced no effect.

I, therefore, conclude that the killing power of ultra-violet light begins to decrease at about 2800, at first very gradually, reaches one-half of its value at about 2870, one-tenth of its value at 2950, and less than one one-hundredth at 2990. Text-fig. 1 gives a sketch of the probable form of the abiotic curve. As far as I can judge equal intensities produced equal effects in the region 2100 to 2800. If there is a maximum in this region it is at the most only slight and would be in the neighborhood of 2600.

The relative effect of much shorter exposures is shown in Table IV. These exposures give at the same time further data for determining the relation between quantity of energy and per cent of organisms killed. This relation, most likely an integral of the probability law, must be known in order to derive from the tables the form of the abiotic energy curve of Text-fig. 1.



TEXT-FIG. 1. Graph representing the approximate amount of abiotic energy in ultra-violet light as referred to the typhoid bacillus.

TABLE IV.

Short Exposures to the Iron Spark.

Time.	Wave-length.	
	2385-2410 Å. u.	2580-2610 Å. u.
sec.		
60	14, 16, 14, 12	1, 5, 6
50		2
40		3, 3, 2
30	36, 24, 12	5, 4, 5
20		11, 7, 8, 4, 10
15	72, 50, 42	
10		12, 25, 11, 12, 17, 40

The Wratten and Wainwright panchromatic plate requires an exposure of about $\frac{1}{4}$ second under these conditions. At 2600 a 95 per cent sterilization of a *Bacillus typhosus* suspension is secured in about 200 times this length of time. The typhoid organism there-

fore compares very well with the slow silver papers in degree but not in range of sensitivity.

In similar but less extensive experiments I have found *Staphylococcus aureus* to behave exactly like *Bacillus typhosus*.

CONCLUSIONS.

Typhoid bacilli are about one two-hundredth as sensitive to ultra-violet light of wave-lengths 2100 to 2800 Ångström units as is the photographic plate. This sensitivity then falls off, decreasing rapidly to almost zero sensitivity at about 2970, the beginning of the sun's spectrum.

I wish to thank Dr. Paul Lewis for suggestions and advice in the arrangement of the experiments.

EXPLANATION OF PLATE 66.

FIG. 1. The iron spark spectrum between 2100 and 3200 Ångström units. The upper band is the spectrum with a narrow slit, the lower band, the spectrum with a wide slit as for bacterial exposures. Below 2320 Ångström units the faintness of the spectrum is real. Below 2200 the increasing faintness is due to the plate.

FIG. 1.

(Newcomer: Abiotic action of ultra-violet light.)

CHANGES IN THE PERIPHERAL BLOOD CONSEQUENT UPON THE DIVERSION OF THE SPLENIC BLOOD INTO THE GENERAL CIRCULATION.

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PLATE 67.

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Studies of the peripheral circulation after Eck fistula in dogs have been reported, but on account of the fact that by diverting the portal blood into the inferior vena cava one has included blood from all the tributaries of the portal vein, it seemed advisable to make a thorough and careful study after the splenic blood alone had been thrown into the general circulation.

HISTORICAL.

A review of the literature revealed the fact that numerous investigators had attempted to discover the function of the spleen by means of a study of the splenic circulation itself. Before Eck, in 1877, devised his method of diverting the portal and splenic blood into the general circulation, Dobson, Warner, Eagle, Hargrave, and others had advanced various theories in regard to the function of the spleen that were based upon the distribution of the splenic circulation as determined by anatomical dissection in animals and in human cases. Ecker and Gerlach studied the blood corpuscles in relation to the spleen. And in 1851 Funke made physiological and chemical experiments upon the blood of the splenic artery and vein. Following the publication of the experimental method and the results of diverting the portal circulation into the inferior vena cava by Eck in 1877, extensive studies have been made on animals with an Eck fistula. That most of these studies were physiological and chemical is evident from reading the reports of Hahn, Massen, Nencki, and Pawlow, Kotliar, Hedon and Delezenne, de Filippi, Schupfer, Magnanimi, Salaskin and Zaleski, Popelski, Bielka von Karltreu, Tansini, Rothberger and Winterberg, Hawk, Macleod, Sweet and Levene, Voegtlin and Bernheim, Towles and Voegtlin, Matthews, Fischler and Kossow, and Whipple and Hooper. A number of different methods of per-

forming the anastomosis between the portal vein and the inferior vena cava, with the purpose in view of improving the operative technique, have been reported by Pawlow and Massen in 1893, by Queirolo in 1895, by Sweet and Herrick in 1905, by Guleke in 1906, by Fischler and Schröder in 1909, by Bernheim, Homans, and Voegtlin in 1910, by Frouin and Jéanne in 1911, by Franke in 1912, by Jeger and Reschad and du Bois-Reymond in 1913, and by Peet in 1914.

A study of the peripheral blood after Eck fistula was made by Nassau in 1914, who concluded from his investigation that the blood picture of dogs with Eck fistula did not deviate from the normal, neither did the tissue intoxication or the

TABLE I.

Author.	Red blood count.	White blood count.	Percentage of white blood count.		
			Polymorphonuclear neutrophils.	Polymorphonuclear eosinophils.	Mononuclears.
			per cent	*per cent*	*per cent*
Schittenhelm, Weichardt, and Grisshammer.	5,500,000–6,000,000	9,000–12,000	63–77	3–5	17–25
Kleineberger.	7,225,000	10,000	77.3	4.2	8.6* 7.0†
Nassau.	5,400,000–7,300,000	5,000–13,400	63–79 (72)	3.5–10	13–25.5 (18)* 2.67–6.6 (5.2)‡
Musser and Krumbhaar.	4,630,000–7,760,000	15,923	66	5	22* 6.8†

* Small mononuclears.

† Large mononuclears.

‡ Large mononuclears and transitionals.

anaphylactic reaction. In dogs with Eck fistula he was unable after repeated injections of albumin to demonstrate the classical blood picture of anaphylactic shock. Also dogs with Eck fistula and ligation of the common bile duct became jaundiced and died after a short time in the course of the jaundice, and the red blood cell count dropped within 2 to 6 days approximately to two million cells. And finally, the resistance of the red blood cells of Eck fistula dogs against hypotonic common salt solution was unchanged. Table I shows the normal blood count of dogs as determined by Nassau and others.

There was also quite a variation in the blood counts of normal dogs, as was given in the reports of observations by Busch and Van Bergen, Dawson, Goodall,

Biedel and Decastella, Tallquist and Reckzeh.[1] It was therefore evident that the most accurate method to employ in order to avoid this great variation in animals would be to determine the control blood counts of each dog that was studied.

A search through the literature revealed only one instance in which a study of the peripheral circulation after the splenic blood alone had been diverted into the general circulation is given; and the authors, Krumbhaar, Musser, and Peet, reported difficulty in doing the anastomosis between the splenic vein and the inferior vena cava. Following the operation they noted an immediate polymorphonuclear leukocytosis and transitional rise, which was prolonged by a more persistent lymphocytosis and eosinophilia, as in splenectomized animals; also a moderate anemia and slight increased resistance of the red blood cells to hypotonic salt solution; a postoperative loss of weight followed by an increased weight, as in splenectomized animals; abdominal adhesions and perisplenitis; and the urine free from bile.

EXPERIMENTAL.

Because of the rather uniform results obtained in the five large dogs that were studied and the simple method that was used to divert the splenic blood into the general circulation, it seemed advisable to report the following experiments. The method employed in the present problem consisted in the study of five dogs over a sufficiently long period before operation in order to establish accurate controls for each dog; and after the splenic blood had been diverted into the general circulation this study was repeated over a period varying from 1 to 4 months' duration in the different animals. In addition, 2 weeks before the end of the experiment the dogs were vitally stained to a maximum with trypan blue, and finally they were killed in order to determine the gross and histological findings.

At the time of operation, with the animals under ether anesthesia, the spleen was first measured through a left rectus incision and a small histological control section removed. After the splenic and the left renal vein had been dissected out, and the common trunk of the splenic vein had been divided between double ligatures, a lateral anastomosis was made between the renal and splenic veins, using fine beaded black silk, oil technique, and a Peet three-bladed, spring-jawed blood vessel clamp; and lastly, all possible collateral circulation

[1] These authors are quoted by Musser and Krumbhaar in their article on studies of the blood of normal dogs (1914–16).

with the spleen was divided, especially the communicating branches between the spleen and the stomach (Fig. 1).

The animals recovered rapidly from operation and the wounds healed by first intention in every case. The dogs kept in splendid general condition throughout the experiment and several of them were operated upon a second and a third time in order to verify intra-

TABLE II.

*General Blood and Weight Table of Dog 1.**

Date.	Average of two white blood counts.	Average of two red blood counts.	Hemoglobin (Sahli).	Weight.
1917			*per cent*	*lbs.*
Apr. 11	17,180	8,424,000	83	$31\frac{3}{8}$
" 12	14,700	8,228,000	86	$30\frac{3}{8}$
" 13	16,100	7,856,000	87	$31\frac{1}{2}$
" 21	Date of operation.			
" 22	45,200	8,644,000	93	$28\frac{5}{8}$
" 23	36,900	7,812,000	83	$28\frac{7}{8}$
" 24	30,540	7,952,000	83	29
" 25	31,460	7,908,000	86	$29\frac{1}{4}$
" 26	21,720	8,164,000	81	29
" 27	19,220	8,084,000	89	$29\frac{3}{8}$
" 28	33,480	8,000,000	90	28
" 30	27,070	8,384,000	87	$28\frac{1}{8}$
May 2	24,560	7,748,000	84	28
" 4	25,980	7,864,000	78	$27\frac{3}{4}$
" 7	22,580	7,890,000	79	28
" 9	15,120	7,644,000	91	$27\frac{3}{4}$
" 11	19,020	7,921,000	83	$27\frac{7}{8}$
" 14	20,340	7,196,000	85	28
" 19	16,040	7,616,000	86	$30\frac{1}{8}$
" 21	16,960	7,732,000	83	30

* See Text-fig. 1 for graphic chart.

abdominal conditions, with the result that no collateral circulation and no intraabdominal adhesions were found, and the anastomosis was working. Fig. 1 clearly shows the anatomical arrangement and the final operative result which occurred in every instance. Aside from a moderate temporary loss of weight after operation, nothing unusual was noted in the condition or actions of the animals at any

time. The animals were never jaundiced and the urine and stools were negative.

Tables II to V and Text-figs. 1 to 4 show most clearly and briefly the general condition of the animals and the changes in the peripheral blood. Because of lack of space and also since all the animals showed essentially the same changes, only a few tables and charts from two

TABLE III.

*General Blood and Weight Table of Dog 2.**

Date.	Average of two white blood counts.	Average of two red blood counts.	Hemoglobin (Sahli).	Weight.
1917			*per cent*	*lbs.*
Mar. 2	16,580	7,620,000	88	28⅜
" 3	18,790	7,300,000	80	28⅛
" 4	19,800	7,204,000	80	27½
" 16	Date of operation.			
" 17	28,580	7,072,000	75	27⅜
" 18	27,420	6,768,000	70	26 1/10
" 19	22,240	7,252,000	73	25¾
" 20	18,060	6,732,000	78	25⅜
" 21	14,080	6,784,000	83	27¼
" 23	15,760	6,280,000	72	26⅝
" 26	10,100	6,632,000	76	25¾
" 30	10,760	7,533,000	82	27⅛
Apr. 2	12,820	7,320,000	82	26¾
" 9	12,060	8,115,000	84	26⅝
" 16	9,000	7,956,000	80	25⅞
" 23	11,850	7,556,000	90	26¼
" 30	11,700	7,480,000	88	26⅜
May 7	12,000	7,636,000	90	26⅜
" 14	12,080	7,144,000	89	26⅜
" 21	11,460	7,440,000	86	26⅜

* See Text-fig. 3 for graphic chart.

dogs are included in the present article. There was an immediate high postoperative rise of leukocytes without any especial change in the hemoglobin or the red blood cells, which had a normal appearance in fresh blood films. The white blood cells seemed to react more acutely and sensitively than one would have expected, in an uninfected wound, and out of all proportion to the splendid post-

TABLE IV.

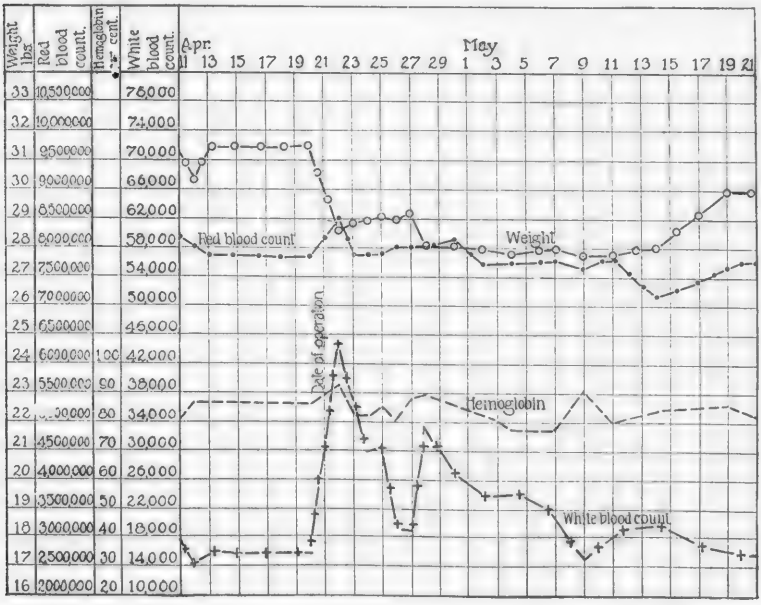
Average Daily Differential Percentage White Blood Count Based upon Three Differential Counts of 250 Cells Each.

Date.	Polymorphonuclear neutrophils.	Polymorphonuclear basophils.	Polymorphonuclear eosinophils.	Small mononuclears.	Large mononuclears.	Transitional cells.	Unidentified.	Smudges.
			Dog 1.*					
1917	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Apr. 11	51.33	0.53	21.6	18.13	1.73	3.46	0.26	2.93
" 12	52.00	0.4	20.53	20.4	1.46	4.13	0.26	0.26
" 13	43.06	0.53	24.4	19.73	1.73	3.73	0.4	6.4
" 21	Date of operation.							
" 22	90.33	0.0	0.53	4.53	0.4	3.6	0.0	0.53
" 23	61.13	0.07	21.33	10.27	0.4	5.73	0.07	2.27
" 24	61.25	0.0	17.5	8.8	1.4	9.2	0.1	1.1
" 25	54.0	0.0	18.3	15.1	1.9	9.7	0.1	0.9
" 26	47.2	0.1	20.2	18.9	2.3	9.8	0.1	1.4
" 27	47.10	0.0	13.7	14.9	2.5	10.0	0.3	5.3
" 28	52.90	0.0	17.0	17.6	1.5	9.9	0.1	2.1
" 30	56.2	0.2	11.8	16.1	1.8	8.5	0.1	1.9
May 2	61.90	0.1	8.6	13.7	2.8	8.2	0.1	4.6
" 4	57.3	0.0	9.8	21.0	2.7	8.4	0.2	0.5
" 7	64.53	0.0	7.3	19.07	0.8	6.53	0.0	1.73
" 9	44.0	0.0	19.73	29.2	1.83	7.6	0.0	0.93
" 11	49.2	0.0	20.27	23.47	1.2	5.3	0.13	0.4
" 14	52.53	0.0	20.43	18.47	0.93	6.27	0.0	1.3
" 19	50.53	0.0	21.3	20.53	1.2	5.47	0.0	0.93
" 21	53.53	0.0	17.2	21.07	1.2	6.13	0.0	0.53
			Dog 2.†					
Mar. 2	57.1	0.1	17.5	13.9	2.3	5.9	0.3	2.9
" 3	63.73	0.13	9.33	17.86	2.4	5.2	0.26	1.2
" 4	64.0	0.0	18.4	11.46	0.93	3.86	0.13	1.2
" 16	Date of operation.							
" 17	88.93	0.0	1.6	2.8	1.06	3.6	0.0	2.0
" 18	78.4	0.13	7.73	6.93	0.93	6.93	0.26	2.0
" 19	71.2	0.13	8.93	8.4	0.53	9.6	0.13	1.06
" 20	64.4	0.0	16.6	3.8	0.9	12.4	0.0	1.73
" 21	56.6	0.0	13.6	9.86	1.33	14.53	0.13	3.86
" 23	64.26	0.0	10.26	7.2	0.93	14.00	0.0	3.33
" 26	52.4	0.0	7.83	17.46	2.8	16.8	0.13	2.53
" 30	56.8	0.0	9.6	15.33	2.66	12.93	0.26	2.93
Apr. 2	48.26	0.0	16.4	18.00	2.4	11.83	0.53	2.53
" 9	58.4	0.26	20.53	28.53	2.66	11.6	0.0	1.33
" 16	42.73	0.13	21.06	19.73	2.0	12.4	0.13	2.13
" 23	40.8	0.4	28.3	16.8	2.26	9.86	0.26	0.93
" 30	41.46	0.4	28.4	14.26	2.8	10.66	0.1	1.86
May 7	41.06	0.0	23.6	19.6	1.6	9.33	0.0	1.46
" 14	44.4	0.0	24.4	18.8	2.53	8.93	0.13	0.8
" 21	49.6	0.0	26.93	13.73	1.6	7.3	0.0	0.8

* See Text-fig. 2 for graphic chart.

† See Text-fig. 4 for graphic chart.

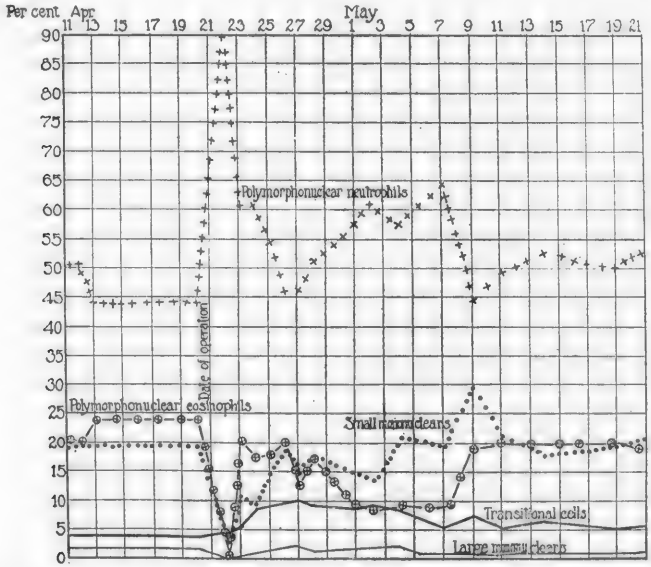
operative condition of the animals. The percentage differential white blood counts demonstrated that the great increase in leukocytes was due to the polymorphonuclear neutrophils, which composed 88 to 95 per cent of the total number of cells, and that came down to their preoperative percentage within from 2 to 7 days. But the most notable change in the peripheral blood was in the transitional white blood cells, which were immediately increased after operation,



TEXT-FIG. 1. General blood and weight chart of Dog 1 (Table II).

although they did not reach their maximum number until after 3 to 5 days. These cells, which were characterized by a large horseshoe-shaped nucleus, remained increased in number over months, and only very slowly approached their preoperative number, a fact which was probably accounted for by a gradual readjustment and decreased production to meet a lessened need in the peripheral circulation, although these transitional cells never did return quite to the preopera-

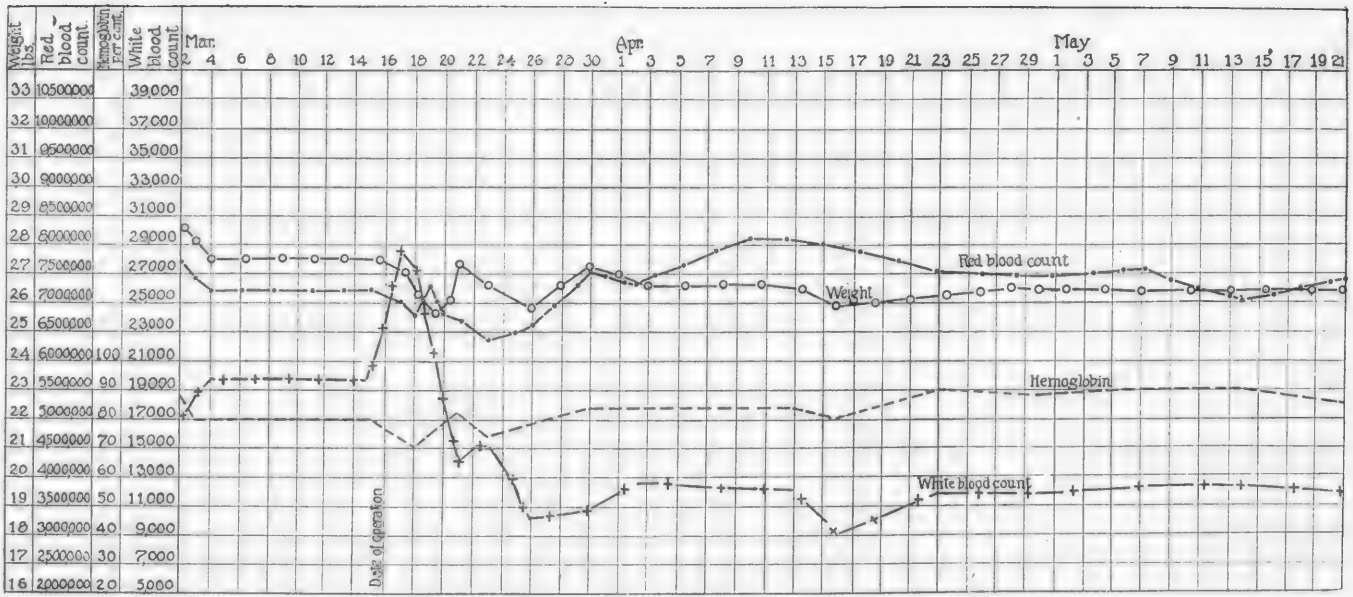
tive percentage during the course of the experiment. In several of the animals there was an immediate increase in the large mononuclear cells and so called smudges, which were probably either large mononuclear or transitional cells. The small mononuclear cells and the eosinophils were relatively decreased immediately after operation, but subsequently gradually increased to their preoperative percentage. In three animals there was a late rise of small mononuclear and eosinophilic cells with a relative decrease in the polymorphonuclear neutro-



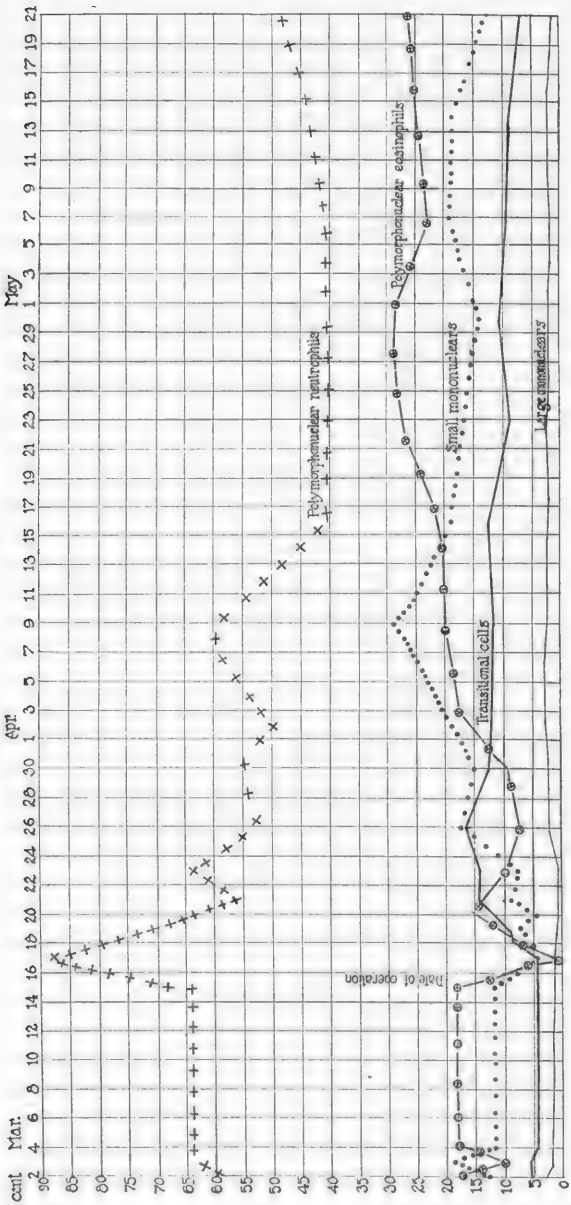
TEXT-FIG. 2. Percentage differential white blood count of Dog 1 (Table IV).

phils, and in one dog there was a late increase of small mononuclear cells alone. Naegeli gives drawings of the various types of blood cells.

After periods varying in duration from 1 to 4 months, subsequent to the first operation, the animals were killed in order to verify the splenorenal anastomosis and to note any pathological changes. The dogs were in splendid general condition, well nourished, active and healthy looking, and the tissues were well stained with trypan blue. The following procedures were carried out at the time that the ani-



TEXT-FIG. 3. General blood and weight chart of Dog 2 (Table III).



TEXT-FIG. 4. Percentage differential white blood count of Dog 2 (Table IV).

TABLE V.

Actual Number of Various White Blood Cells Per Cc.

Date.	Polymorphonuclear neutrophils.	Polymorphonuclear basophils.	Polymorphonuclear eosinophils.	Small mononuclears.	Large mononuclears.	Transitional cells.	Unidentified.	Smudges.
				Dog 1.				
1917								
Apr. 11	8,813.0	86.0	3,711.0	3,109.0	292.0	584.0	52.0	498.0
" 12	7,644.0	59.9	3,014.0	2,999.0	215.0	603.0	29.0	29.0
" 13	6,923.0	80.5	3,928.4	3,171.7	273.7	595.7	64.4	1,030.4
" 21	Date of operation.							
" 22	40,815.6	0.0	226.0	2,034.0	180.8	1,627.2	0.0	226.0
" 23	22,545.90	36.9	7,859.7	3,800.7	147.6	2,114.4	36.9	848.7
" 24	18,690.5	0.0	5,344.5	2,686.5	427.5	2,809.68	30.54	335.9
" 25	16,988.4	0.0	5,757.18	4,744.46	597.74	3,051.6	31.46	283.14
" 26	10,251.84	21.72	4,387.44	4,005.08	499.56	2,128.56	21.72	304.08
" 27	9,052.62	0.0	2,633.19	2,863.78	480.50	1,922.00	57.66	1,018.66
" 28	17,710.92	0.0	5,691.60	5,892.48	502.20	3,314.52	33.48	703.08
" 30	15,353.84	54.64	3,223.76	4,398.52	491.76	2,322.20	27.32	529.08
May 2	15,202.64	50.00	2,112.16	3,364.72	687.67	2,013.92	24.56	1,129.96
" 4	14,786.54	24.56	2,566.04	5,455.80	701.46	2,183.32	51.98	129.9
" 7	14,564.10	0.0	1,648.34	4,312.78	180.64	1,467.70	0.0	383.86
" 9	6,652.80	0.0	2,978.64	4,415.04	272.60	1,149.12	0.0	136.08
" 11	9,357.84	0.0	3,861.06	4,569.70	228.24	1,008.06	0.0	76.08
" 14	10,678.50	0.0	4,088.34	3,762.90	183.06	1,281.42	0.0	264.42
" 19	8,100.20	0.0	3,416.52	3,288.20	192.48	882.20	0.0	144.36
" 21	9,073.60	0.0	2,917.12	3,578.56	203.52	1,034.56	0.0	84.80
				Dog 2.				
Mar. 2	9,467.18	16.58	2,901.5	2,314.62	381.34	978.25	49.74	480.82
" 3	11,968.93	18.79	1,747.47	3,360.41	450.96	977.00	56.37	225.48
" 4	12,672.0	0.0	3,643.20	2,277.00	178.20	772.20	19.80	237.60
" 16	Date of operation.							
" 17	25,457.62	0.0	457.28	800.24	302.94	1,028.88	0.0	591.60
" 18	21,497.28	27.42	2,111.34	1,891.48	246.78	1,891.48	82.26	548.4
" 19	15,834.88	22.24	1,979.36	1,868.16	111.20	2,135.04	22.24	244.64
" 20	11,630.64	0.0	2,997.96	686.2	162.54	2,239.44	0.0	307.92
" 21	7,969.28	0.0	1,914.88	1,393.92	183.04	2,041.60	14.08	549.12
" 23	10,143.68	0.0	1,633.28	1,134.72	14.84	2,206.40	0.0	630.08
" 26	5,292.40	0.0	787.80	1,767.50	282.80	1,696.80	10.10	252.50
" 30	6,111.68	0.0	1,032.96	2,646.28	290.52	1,388.04	32.28	312.04
Apr. 2	6,192.06	0.0	2,102.48	2,307.60	307.68	1,512.76	64.10	320.50
" 9	7,043.04	36.8	2,472.30	3,437.10	325.62	1,398.96	0.0	156.78
" 16	3,845.7	9.0	1,899.00	1,773.00	180.00	1,116.00	9.0	189.00
" 23	4,834.80	47.4	3,353.55	1,990.80	272.55	1,173.15	35.55	106.65
" 30	4,855.50	46.8	3,322.8	1,673.1	327.6	1,251.90	11.7	222.3
May 7	4,932.00	0.0	2,832.00	2,352.00	192.00	1,116.00	0.0	180.00
" 14	5,363.52	0.0	2,947.52	2,271.04	302.00	1,075.12	12.0	96.64
" 21	5,684.16	0.0	3,082.74	1,570.02	183.36	836.58	0.0	96.64

mals were killed. Under ether anesthesia, the spleen was measured and found to be either the same size or slightly larger than at the first operation. After the splenic and renal vessels were dissected out, the anastomosis was proved to be working in every case by clamping the renal vein beyond the anastomosis, which caused the spleen to swell. A probe could be passed through the anastomosis into all the various contributing branches. No gross changes were evident in the kidneys or renal veins, and there were no unusual intraabdominal adhesions and no collateral circulation established with the spleen. In two dogs the surface of the spleen was smooth and healthy in appearance. In three instances it was somewhat grayish and slightly thickened. The consistency of the spleen seemed to be the same as at the time of the first operation. In one animal the thyroid was about twice the size of those of the other dogs. In two cases the thymus was large. Otherwise, there were no gross changes in any of the organs or tissues. The gall bladder was always full of bile, and the liver seemed to be of usual size and consistency. In each instance the portal and splenic veins were tied off at several places while filled with blood, and removed for histological study. This would seem to offer a possible method of studying the blood by histological sections.

The histological study of control sections of the spleen which were removed at the time of the first operation showed them to be similar to the sections that were removed at the end of the experiment, except for a slight active congestion in the later specimens. This active congestion was the result of the method of testing the function of the anastomosis at the time that the animals were killed, and hence had nothing to do with the experimental results. There was no increase in connective tissue and the Malpighian corpuscles appeared as in the control sections. In several of the animals the cells about the portal spaces of the liver stained more deeply than the paler cells in the rest of the liver lobules. Otherwise, no changes were noted in the liver. Considerable pigment was scattered throughout the lung tissue as has been frequently observed in city-bred dogs. A routine examination of histological sections of all the remaining tissues and organs demonstrated no noteworthy changes. Special histological

studies which will be reported upon in a subsequent article are now being made upon the vitally stained spleen and other tissues.

SUMMARY.

1. The principal change in the peripheral blood consisted in the prolonged increase in number of the transitional white blood cells, an active brief stimulation of the polymorphonuclear neutrophils, which were later relatively decreased in number in four cases, in three dogs a late rise in number of the mononuclear and eosinophilic cells, and in one dog of mononuclear cells alone.

2. The normally high differential percentage count of polymorphonuclear eosinophils in dogs would be expected on account of the numerous parasitic infections which they usually have.

3. The splenorenal venous anastomosis offered a simple and satisfactory method of diverting the splenic blood into the general circulation because it was easy and produced no gross abnormal intra-abdominal changes, and the vessels normally lay parallel to each other and were readily approximated without tension.

4. The operation was successful in every case.

5. The animals did very well after operation and were healthy and active.

6. No noteworthy histological changes were observed in any of the organs or tissues.

7. There was no essential change in bile production that could be detected by jaundice.

8. The urine and stools showed no changes.

Finally, I wish to express my appreciation to Dr. Emil Goetsch of the Johns Hopkins University for his assistance and interest.

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EXPLANATION OF PLATE 67.

FIG. 1. The splenorenal venous anastomosis as it appeared at the end of the experiment. The common splenic vein was divided; the gastrosplenic veins were also divided.

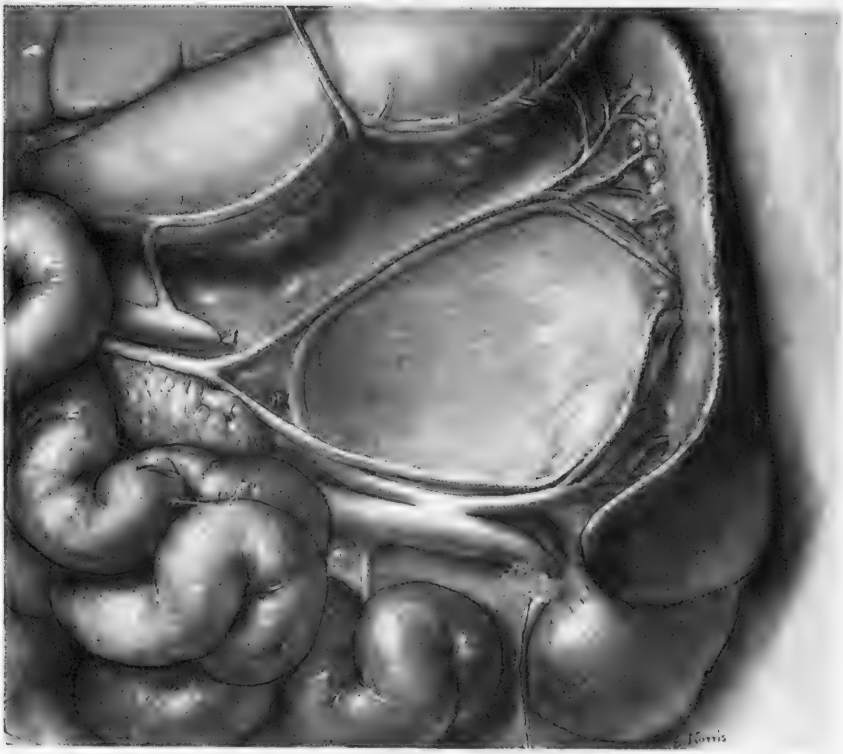

FIG. 1.

IDENTITY OF THE TOXINS OF DIFFERENT STRAINS OF BACILLUS WELCHII AND FACTORS INFLUENCING THEIR PRODUCTION IN VITRO.

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(Received for publication, September 1, 1917.)

It has been reported in an earlier publication[1] that under suitable cultural conditions *Bacillus welchii* produces an exotoxin which is comparable in its physical and biological properties with diphtheria and tetanus toxins. The original study was limited to five strains of the organism, all of which yielded toxins which were qualitatively identical although differing quantitatively. The identity of the various toxins was determined by the observation that an antitoxin produced with a toxin from any strain neutralized the toxins from all the other strains. Moreover, it was found that such a monovalent antitoxin would protect against and control infections with any of the strains included in the study. The small number of strains studied and the closely related sources of four of the five made it desirable to extend the experiments before concluding that all strains of *Bacillus welchii* produce the same toxin. From the standpoint of developing a specific therapy, it was essential to know whether one toxin is common to all strains, regardless of their origin. To ascertain this, twenty-two other strains have been collected from widely different sources and studied with reference to the identity of their toxins.

Source of the Strains.

Two of the new strains were part of the cultures isolated during the summer of 1916 on the Western Battle Front by Dr. J. P. Simonds of Chicago. Four strains were obtained from the Pasteur Institute through the kindness of Dr. Carrel. Dr. Carrel also brought us necrotic tissue from two cases of severe gaseous gangrene, from which

[1] Bull, C. G., and Pritchett, I. W., *J. Exp. Med.*, 1917, xxvi, 119.

we isolated the infecting strains. Two strains were isolated from cases of gaseous gangrene occurring in New York City. Five strains were obtained through the kindness of Dr. F. M. Allen from spontaneous gas bacillus infections in diabetic dogs, two others from dog feces, two from human feces, two from market milk, and one from garden soil.

All the strains were obtained in pure culture as indicated by morphological, cultural, and pathogenetic properties, and each strain was tested separately, without animal passage, for artificial toxin production.

Production and Identification of the Toxins.

Each strain was grown in 0.2 per cent glucose muscle broth for 20 hours at 37°C., and the cultures were centrifuged and filtered.[1] The filtrates were tested for toxicity by intramuscular injection in pigeons. The same quantity of filtrate that was used in the toxicity tests was mixed respectively with *Bacillus welchii* antitoxin and an equal amount of normal serum of the same animal. The mixtures were incubated for 30 minutes at 37°C. and then injected into the breast muscles of pigeons. The degree of toxicity of the filtrate and of the serum-filtrate mixtures was calculated from the degree of the local edema and necrosis which ensued and the quantity of filtrate necessary to cause death.

The procedure described established the fact that all the strains produced toxins which were indistinguishable in pathologic effects from those produced by the five strains described in our first publication, and that an antitoxin made with toxin from one of the former strains completely neutralized the toxins from all the new strains. The toxins of the individual members of the series exhibited, however, a wide range of potency; the lethal dose for pigeons on intramuscular inoculation varied from 0.3 cc. to 3 cc. The lesions and toxic effects were, nevertheless, of the same quality. The toxin-producing power of the less active strains was materially increased by animal passage, and an indication was obtained that a direct relation between infectiosity and toxin production exists. However, the latter point demands a more systematic study than we have yet been able to give it.

To summarize, we may state that twenty-two new strains of *Bacillus welchii* have been secured from widely different sources and studied with regard to morphological, cultural, and pathogenetic properties and toxin production. They have by these means been identified as typical members of the *Bacillus welchii* group of bacilli. Moreover, each strain produced a toxin which was qualitatively indistinguishable in physical and biological properties from the toxins produced by any other members of the series, although the different toxins manifested varying degrees of potency.

Up to the present time we have studied twenty-seven strains of *Bacillus welchii* from widely different sources and have found all to produce a toxin in common.

Factors Influencing the Artificial Production of Bacillus welchii Toxin.

In our first publication[1] the following method was given for obtaining the toxin *in vitro*: To plain beef infusion broth in 10 cc. quantities in test-tubes are added several fragments of sterile skeletal muscle of the pigeon or rabbit. The tubes, having been proved sterile, are inoculated with *B. welchii*, overlaid with paraffin oil, and enclosed in a vacuum jar from which the oxygen is then exhausted. After an incubation of from 18 to 24 hours, the contents of the culture tubes are centrifuged and the fluid portion of the culture is passed through a Berkefeld N candle. The filtrate contains the toxin. It was stated also that to obtain the most potent product not more than 0.1 per cent glucose should be present, and that the incubation should not exceed 24 hours.

Since then we have been able to carry out a more extensive and systematic investigation of the influence of various factors on artificial toxin production.

Certain technical precautions are necessary in determining the influence of different factors on the production of the toxin *in vitro* in order to eliminate extraneous influences. In the first place, the culture with which the media are inoculated must be controlled with regard to age, the medium in which it is grown, and the number of artificial generations since animal passage. Then the different lots of medium in which toxin is to be produced for the purpose of comparison should be inoculated simultaneously and with the same quantity of culture. Errors may arise in the process of collecting the filtrates. A portion of the toxin is always held back by the filter-

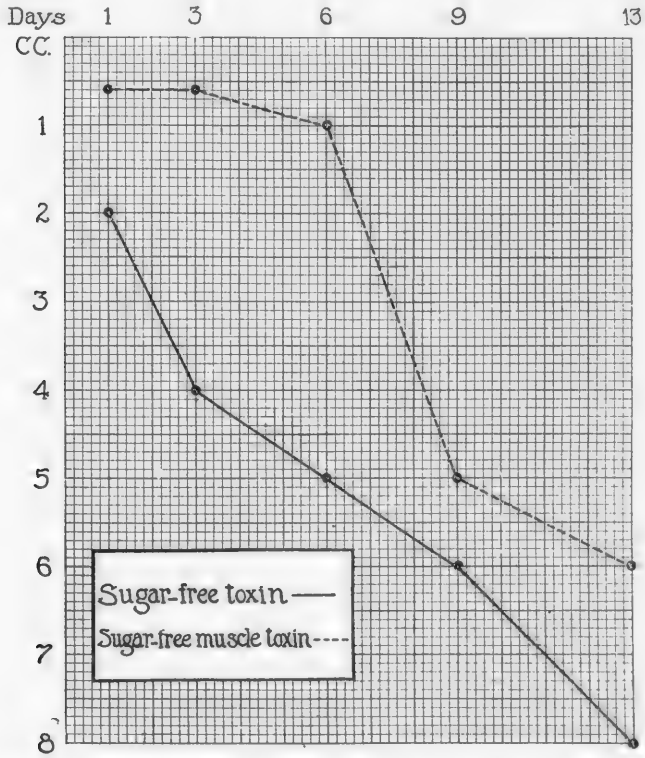
ing candles, and if they are not perfectly clean and open, a large percentage of the toxin may be retained. Under the best conditions different candles seem to retain different amounts of toxin. The following procedure has been followed in this work: A good toxin-producing strain (617 d, Simonds) was used. The strain was of maximum virulence as the result of a number of animal (pigeon) passages. A pigeon was inoculated with an acutely lethal dose of the culture early in the morning of the day preceding the beginning of an experiment. Immediately after the death of the animal, usually late in the afternoon, tubes of plain beef infusion broth were inoculated with small fragments of the infected muscle and incubated at 37° C. over night. The test media were then inoculated with definite quantities of the bouillon cultures, exhausted in a vacuum jar, and incubated in the usual way. At the end of the planned incubation period, the contents of the culture tubes were centrifuged until the supernatant fluid was free of solid matter. The fluid was then syphoned off and filtered. The filters were proved to be open by testing them with sterile distilled water immediately before use and were recleaned as soon as there was evidence of obstruction of the flow of the fluid through them. In this way consistent results were obtained.

In order to test the potency of the toxin, three different quantities of each toxic product were injected into the breast muscles of three pigeons respectively. The degree of toxicity was estimated from the extent of the edema and necrosis, when sublethal amounts were given, and from the minimal lethal dose. In a number of instances, where the degree of toxicity could not be approximately predicted, the largest quantities given did not cause death or even local lesions. This was especially true of the filtrates from the cultures containing the higher percentages of glucose, in which instances only relative values were obtained.

Influence of Fresh Muscle and Incubation Time on the Potency of the Toxin.

We had observed more or less incidentally that the most potent filtrates were obtained after the cultures had been incubated from 18 to 24 hours, and that an extension of the incubation period caused

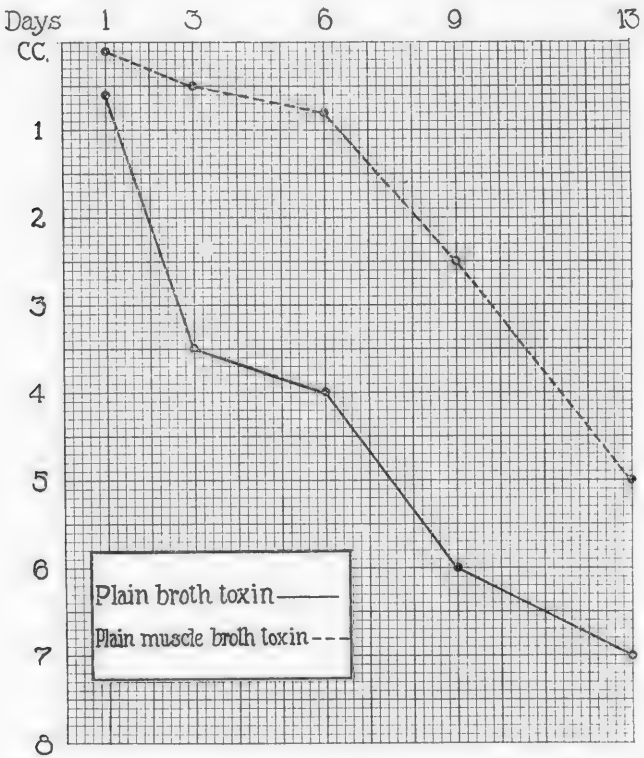
a decrease in toxicity; but the rate and the extent of the decrease in toxicity as the incubation time is prolonged had not been determined. It was, therefore, considered desirable to determine more accurately the relation between incubation time and toxicity. With this end



TEXT-FIG. 1. The media were sugar-free broth and sugar-free broth to which fresh sterile muscle had been added. The tubes were inoculated, freed of oxygen, and incubated in the usual way. The two products are designated as sugar-free toxin and sugar-free muscle toxin respectively.

All the text-figures have the same arrangement. The incubation time of the different specimens of filtrate is represented on the ordinates, while the smallest quantities necessary to produce local lesions and death are represented on the abscissæ. The height of the curves from the base-line represents, therefore, the degree of toxicity.

in view, a variety of media, consisting of various kinds of beef infusion, was inoculated with cultures of *Bacillus welchii* and the filtrates were collected and tested at different intervals, ranging from 16 hours to 13 days.

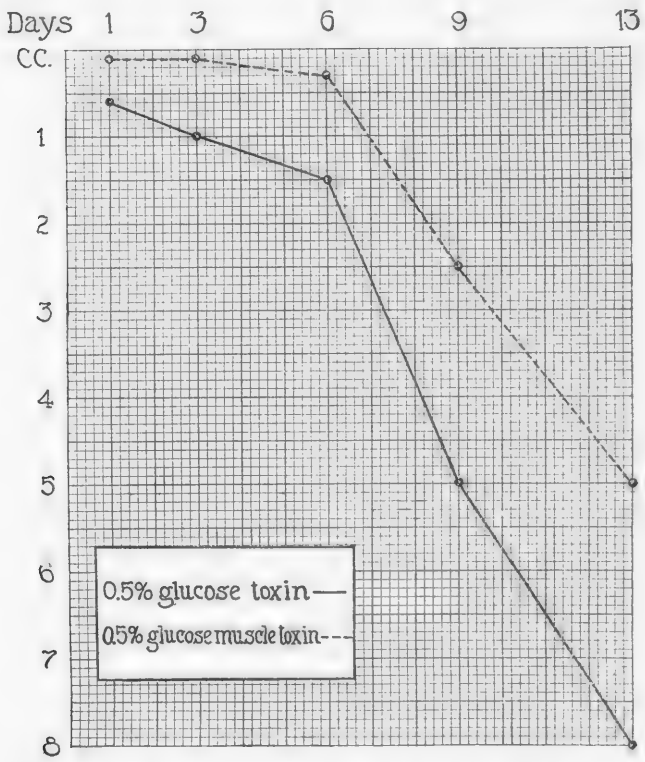


TEXT-FIG. 2. The media were plain broth and plain broth to which sterile muscle had been added. The filtrates are designated as plain broth toxin and as plain muscle broth toxin.

The results obtained may be summarized in the statement that the toxicity of the filtrates is inversely proportional to the incubation time, calculating from the end of the 1st day, and that this general relation obtains independently of the nature of the medium. The

rapidity of the decrease in toxicity, however, is materially influenced by the percentage of glucose in the medium and the presence of raw muscle. The results are presented graphically in Text-figs. 1 to 5.

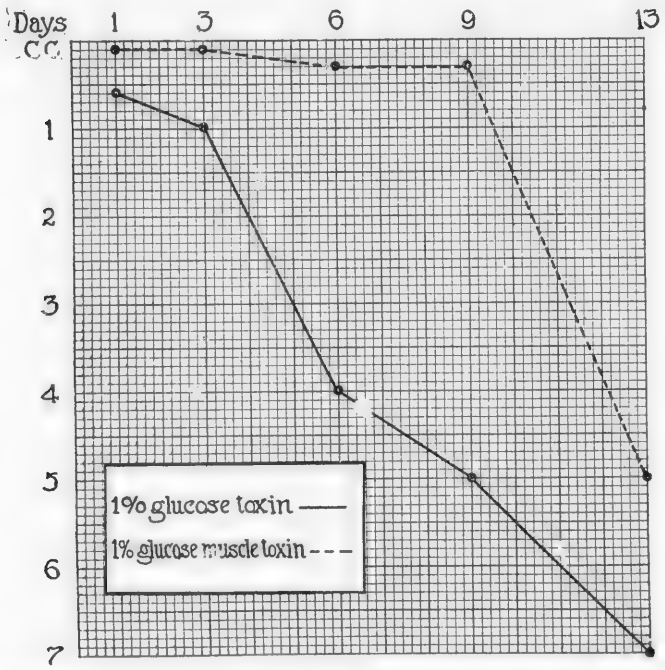
From Text-figs. 1 to 4 it is seen that the toxicity of the filtrates rapidly diminishes as the incubation time is prolonged. The toxicity



TEXT-FIG. 3. The media, 0.5 per cent glucose broth and 0.5 per cent glucose broth plus muscle. The filtrates, 0.5 per cent glucose toxin and 0.5 per cent glucose muscle toxin.

was in every instance highest at the end of the 1st day's incubation. On the 3rd day there was a material decrease in toxicity, especially in the case of the filtrates from the cultures to which muscle had not

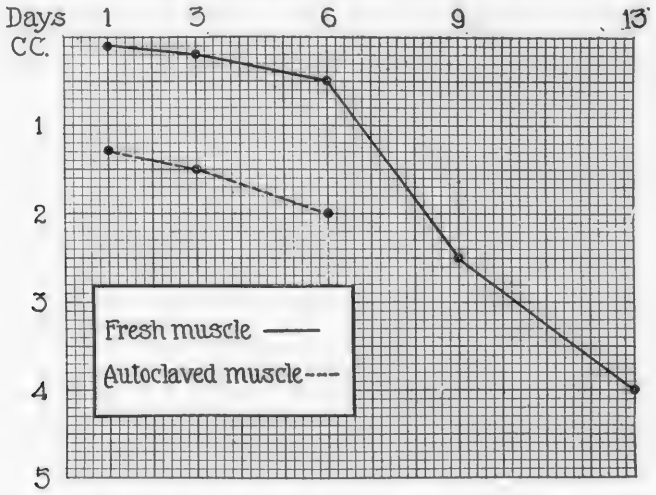
been added. A composite curve of the filtrates from the muscle medium would show that on the 6th day the toxicity had fallen almost 300 per cent. A similar curve of the filtrates from the non-muscle medium would show a decrease in toxicity of 400 per cent. A composite curve including the filtrates from both kinds of media would show a decrease in toxicity of about 1,300 per cent on the 13th



TEXT-FIG. 4. Media, 1 per cent glucose broth and 1 per cent glucose broth plus fresh muscle. Filtrates, 1 per cent glucose toxin and 1 per cent glucose muscle toxin.

day of incubation. It is evident, therefore, that prolonged incubation brings about a destruction of the toxin independently of the nature of the medium, and the most potent toxin is to be obtained at about the end of the 1st day's incubation, the optimum incubation time varying apparently from 18 to 24 hours.

The influence of the muscle upon the potency of the filtrates is very apparent. The 24 hour muscle medium filtrates were in each case about five times as toxic as the corresponding non-muscle filtrates. If the curves had been based on killing power alone, instead of on local effects and killing power, the muscle and non-muscle curves would have been still more widely separated. The toxins formed in the muscle medium seem to have a killing power not possessed by the non-muscle products. Moreover, the non-muscle filtrates lose toxicity more rapidly as the incubation time is prolonged than the



TEXT-FIG. 5. Media, 0.2 per cent glucose broth plus fresh muscle and 0.2 per cent glucose broth plus autoclaved muscle.

muscle filtrates do. The influence of the muscle in the medium is, however, largely a quantitative one. From Text-fig. 5 it is seen that autoclaved muscle will not take the place of fresh muscle.

Glucose, Acidity, and Toxicity.

It is well known that *Bacillus welchii* produces acids, chiefly butyric, under almost any condition of growth, the quantity produced depending largely upon the amount of fermentable sugars present.

Acid production being so prominent and constant, it is natural that students of this organism should have given special attention to that property. Thus, McCampbell[2] made many experiments on the toxic effects of *Bacillus welchii* cultures and their products and concluded that the acids are of prime importance. This conclusion was based on the observation that his neutralized cultural products were inactive, and that butyric acid in a pure state produced identical results with the acid bacillary products. Stewart and West[3] also state that acid by-products are responsible for all the pathologic effects of *Bacillus welchii* infections. Wright[4] holds similar views and has offered theoretical explanations of the way in which the acids operate in bringing about their destructive effects. In our previous paper[1] we have stated that neutralization with sodium hydroxide did not materially alter the pathologic effects of the cultural products, and there was no direct relation between acidity and toxicity. Further observations have been made on this point, and the results are graphically presented in Text-figs. 6 to 10.

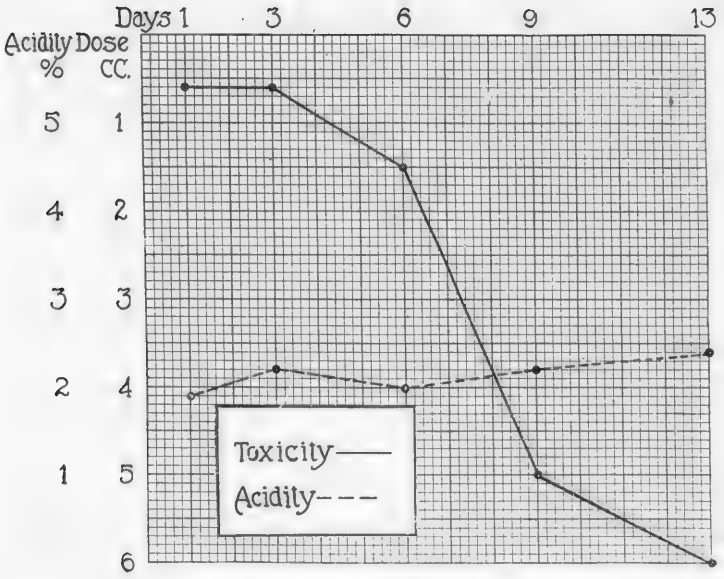
Fragments of fresh rabbit muscle were added to tubes of beef infusion broth containing varying percentages of glucose. A number of tubes of each kind of medium was inoculated with *Bacillus welchii*, a layer of oil added, and the culture tubes were exhausted in a vacuum jar and incubated in the manner already described. Filtrates were collected from a number of the tubes after from 1 to 13 days' incubation and tested for pathologic effects and acidity (Text-figs. 6 to 10).

A study of Text-figs. 6 to 10 discloses the fact that the toxicity of the filtrates is independent of the acidity. The toxic potency of the filtrates rapidly diminishes as the incubation time is prolonged, while the acidity increases or remains constant. In one instance, Text-fig. 9, there was a decrease of 1 per cent in the acidity, but the toxin lost $\frac{24}{25}$ of its potency. When the acidity is high the filtrates show very slight toxic action. Text-fig. 10 illustrates this point. The acidity was 6.8 per cent on the 1st day, rose to 8 per cent by the 3rd day, and remained constant throughout the experiment. The toxicity curve does not represent the actual potency of the filtrate,

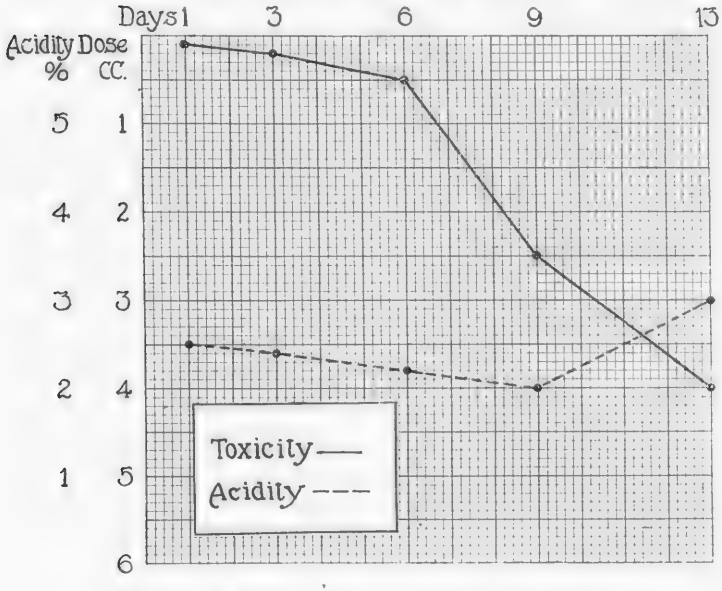
[2] McCampbell, E. F., *J. Infect. Dis.*, 1909, vi, 537.

[3] Stewart, M. W., and West, R., *J. Immunol.*, 1916, i, 189.

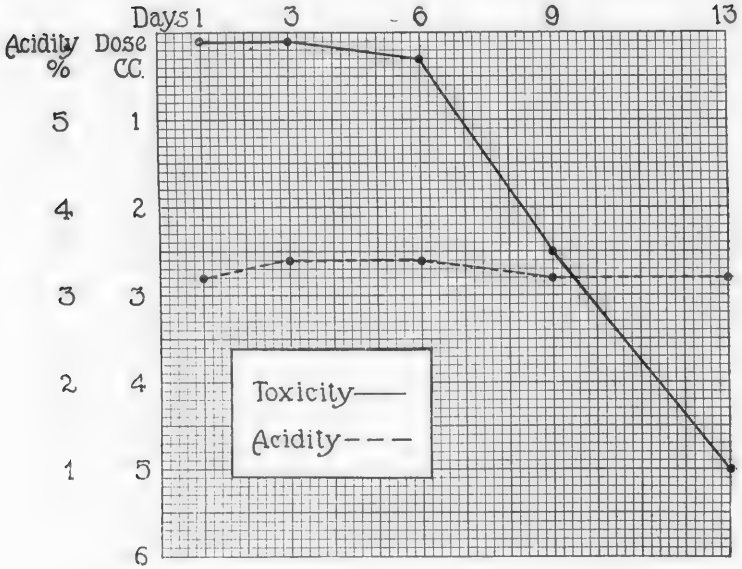
[4] Wright, A. E., *Proc. Roy. Soc. Med.*, 1916–17, x, Occas. Lect., 1.



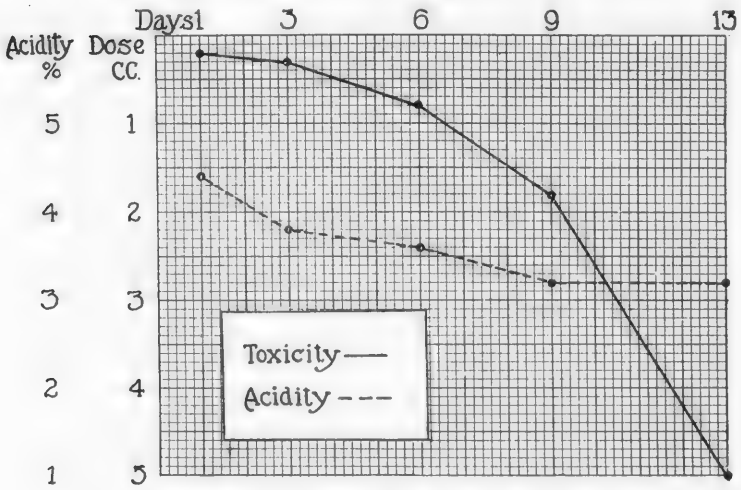
TEXT-FIG. 6. Medium, sugar-free muscle broth.



TEXT-FIG. 7. Medium, 0.2 per cent glucose muscle broth.

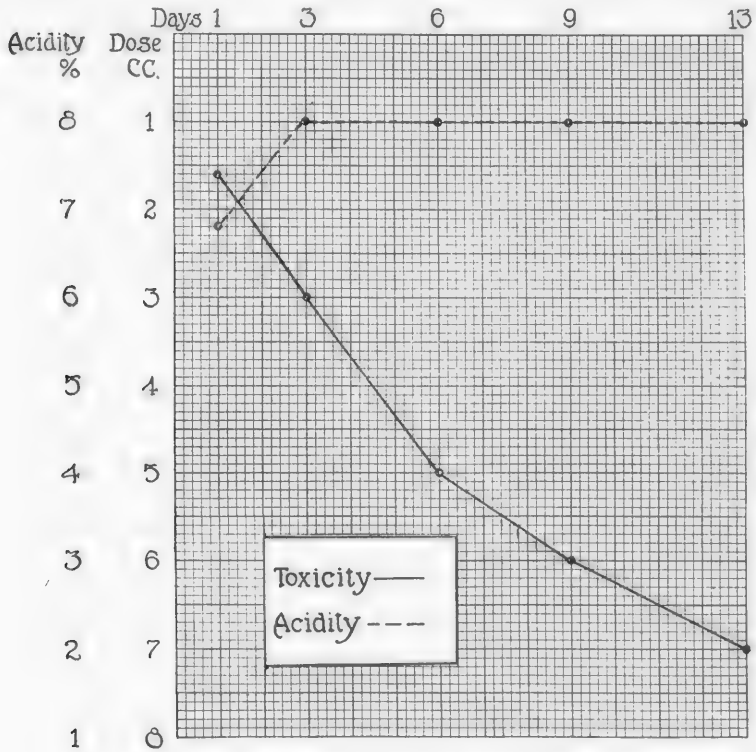


TEXT-FIG. 8. Medium, 0.5 per cent glucose muscle broth.



TEXT-FIG. 9. Medium, 1 per cent glucose muscle broth.

since the quantities used for the animal inoculations were not sufficient to cause death or definite local reactions, the curve being developed from the largest amounts inoculated at the various periods. Beginning with Text-fig. 6, there is a material increase in acidity, which reaches its highest point in Text-fig. 10, while the toxicity increases until Text-fig. 9 is reached, where an abrupt decrease is shown.



TEXT-FIG. 10. Medium, 3 per cent glucose muscle broth.

Text-figs. 6 to 10 may also be used to show the influence of glucose upon toxin production. The glucose content of the medium ranged from 0 to 3 per cent. Since muscle fragments were added to each variety of medium, the percentage of sugar was in each case higher

than is indicated. It is seen that 0.2 per cent glucose causes a material increase in toxicity (Text-figs. 6 and 7). The glucose may be increased to 1 per cent without greatly influencing the potency of the product, while more than 1 per cent has a deleterious effect. The influence of the glucose is, in all probability, non-specific, merely leading to a more luxuriant growth of the bacilli. *As a routine procedure, a 0.2 or 0.3 per cent glucose muscle broth is used for toxin production.*

Review of the Literature.

In our first publication on toxin production by *Bacillus welchii* only brief consideration was given to the work of others on this subject. We propose now to cover the ground somewhat more fully.

In 1904 Kamen[5] reported that in gas bacillus infections in man death occurs under conditions indicating a severe intoxication. He showed also that filtrates from 8 day glucose bouillon cultures of the gas bacillus contained hemolytic substances demonstrable *in vitro*. On the other hand, 10 cc. of the same filtrates caused no lesions on subcutaneous injection in guinea pigs. The author concluded that energetic toxins were not produced in cultures, but that in all probability powerful toxins were produced in the animal body.

Passini's[6] publications on the subject appeared in 1905. He employed a special medium consisting of fresh beef muscle digested with trypsin and sterilized in live steam; to this glucose was added to 1 per cent or more and the air driven out by boiling; or the medium was made by heating the meat-water mixture in an autoclave under from 8 to 9 atmospheres for 1 hour. The medium was then inoculated with the bacilli and incubated for from 14 days to 1 month at 37°C. The filtrates from the cultures were found to be toxic. The toxic effects were manifested immediately on intravenous and intraperitoneal inoculation. When given subcutaneously, edema, necrosis, and sloughing followed. The toxic filtrates resisted heating to 100°C. for 15 minutes, and they were not shown to possess antigenic properties. It is also stated that the filtrates were not toxic if less than 1 per cent glucose was present.

In a general study of the intestinal flora, Metchnikoff gave considerable attention to the gas bacillus.[7] In his case impure cultures were evidently used, since the medium was made with finely chopped beef and tap water, without any attempt at subsequent sterilization. The filtrates from the cultures were found

[5] Kamen, L., *Centr. Bakteriol., Ite Abt., Orig.*, 1904, xxxv, 554.
[6] Passini, F., *Wien. klin. Woch.*, 1905, xviii, 921.
[7] Metchnikoff, E., *Ann. Inst. Pasteur*, 1908, xxii, 951.

to be toxic, the toxicity being greatest after from 2 to 5 days' incubation. A longer incubation period caused a progressive diminution in toxicity. Heating to 100°C. did not reduce the potency of the toxin obtained; moreover, it was not tested for antigenic properties.

Schultze[8] showed that the disappearance of the nuclei in the organs of animals dying of gas bacillus infection was not due to gas formation, and he inferred that the phenomenon was caused by chemical toxic substances formed by the bacilli.

Korentchevsky,[9] working in Metchnikoff's laboratory, found that filtrates from bouillon cultures of the gas bacillus were toxic for rabbits, especially young rabbits. Symptoms of intoxication appeared in from 1 to 3 hours after intravenous and intraperitoneal injections. The chief symptoms were dyspnea, convulsive movements of the head, opisthotonos, clonic convulsions of the extremities, and paralysis. The effects of intramuscular and subcutaneous injections were not determined. Rabbits and dogs were given large quantities (20 cc.) of the filtrates *per rectum* every 2nd or 3rd day for 2½ weeks. The growth of the animals was arrested, and some lost weight. Agglutinins, precipitins, and fixatins were found in the sera of the rabbits. No mention is made of the thermostability, acidity, or antitoxin-producing properties of the filtrates.

Klose[10] studied 135 cases of gas phlegmon, in 39 of which the Fränkel gas bacillus (*B. welchii*) was present. The chief symptom in the cases was intoxication. Toxic substances were demonstrated in the blood sera of 5 cases of gas phlegmon in man. The clinical observations apparently led to the experimental work.

The organism used in the animal experiments was isolated from a foudroyant case of gas phlegmon in man. Toxic substances were demonstrated in the subcutaneous exudates occurring in infected guinea pigs and in Berkefeld filtrates from 5 per cent glucose broth cultures at the end of 14 days' incubation. Subcutaneous injections in guinea pigs gave rise to edema, discoloration, falling of

[8] Schultze, W. H., *Virchow's Arch. path. Anat.*, 1908, cxciii, 419.

[9] Korentchevsky, W., *Ann. Inst. Pasteur*, 1909, xxiii, 91.

[10] After our first publication on *B. welchii* toxin and antitoxin appeared, our attention was called to an abstract in the *Chemical Abstracts*, July 20, 1917, of an article by F. Klose entitled: "Ueber Toxin- und Antitoxinversuche mit dem Fränkelschen Gasbrandbacillus." This abstract was made by Julian H. Lewis from an abstract which appeared in the *Chem. Zentr.*, 1916, ii, 24. The original article appeared in the *Münch. med. Woch.*, 1916, lxiii, 723. This journal could not be found in any of the libraries in New York City or elsewhere in the United States. The abstracts contained only a few summary statements without any experimental details, and no idea of the nature of the work could be formed. The original article has, however, recently come to our hands through the kindness of Dr. J. E. J. King, who returned from Germany in April after rupture of diplomatic relations between the United States and Germany. Because of this accidental circumstance, it is possible to consider the work here.

hair, necrosis, and sloughing; intraperitoneal and intravenous injections caused immediate symptoms, tremors, failure of respiration, and death. The toxic substances were quite thermostable, since 80°C. for 1 hour did not reduce their toxic effects. A horse was immunized as follows: 2,415 cc. of filtrate from 5 day 5 per cent glucose broth cultures were given intravenously during 17½ weeks and after a rest period of 6 weeks, 1,780 cc. of filtrate were given during 6½ weeks. The immune serum protected guinea pigs against three lethal doses of bacilli; 2 cc. of serum were injected at the site of infection 24 hours previously. Infections were controlled by giving the immune serum 2 hours after the guinea pig had been infected.

We have made repeated attempts to produce *Bacillus welchii* toxin artificially according to Klose's method. The filtrates were collected both after 5 days' and 14 days' incubation. Large quantities (5 cc.) of the filtrates failed to cause death or necrosis on intramuscular injection in guinea pigs and pigeons. There was, in some instances, a slight tumefaction of the tissues at the point of inoculation. In the light of these results it is somewhat difficult to explain the pathologic effects of Klose's filtrates. The neutralization and protection experiments, on the other hand, can be readily explained by the fact, observed by us, that a certain number of horses possess natural *Bacillus welchii* antitoxin. Klose did not determine the natural antitoxin content of his horse's serum.

The work of Weinberg and Séguin[11] was referred to in our first publication. These authors made numerous reports on the production of specific antitoxic sera for the bacillus of malignant edema (*vibrion septique*) and for *B. œdematiens* (Weinberg). They reported also that a potent specific antibacterial serum had been produced by immunizing horses with living cultures of *B. welchii* (*B. perfringens*). Frequent mention is made of a *B. perfringens* toxin, but nothing is stated concerning its preparation, or physical or biological properties, but a definite statement is made that it has not been found possible to prepare an antitoxic serum for the organism. Finally, these authors have recently reported on the clinical application of their antimicrobial serum[12] for *B. perfringens* (*B. welchii*) infection.

SUMMARY.

Twenty-two additional strains of *Bacillus welchii* have been collected from widely different sources and tested with regard to toxin

[11] Weinberg, M., *Proc. Roy. Soc. Med.*, 1916, ix, Occas. Lect., 119.
[12] Weinberg, M., and Séguin, P., *Compt. rend. Acad.*, 1917, clxv, 199.

production. Each strain produces a toxin which, on animal inoculation, gives rise to lesions comparable in every respect to those produced by the toxins previously reported on,[1] and each toxin was neutralized by an immune (antitoxic) serum produced with one of the former toxins. The toxins obtained from the several individual strains varied in potency, the lethal dose ranging from 0.3 to 3 cc.

Experiments have been made to determine the influence of fresh muscle and glucose on toxin production and the relation of acidity to toxicity in the filtrates. It has been found that the addition of fresh muscle to the medium increases the potency of the toxin fivefold. Autoclaved muscle is without effect. Beef infusion broth containing 0.2 to 1 per cent glucose gives a more potent product than sugar-free broth, while when higher percentages are employed the toxin production is lowered. There is no direct relation between acidity and toxicity, the most acid products manifesting little or no toxic action. In every medium used for culture the potency of the filtrates rapidly diminished after 24 hours' incubation, while the acidity increased or remained constant. The exception to this rule has been pointed out.

The most active toxin is obtained by growing a virulent strain of the bacilli in a 0.2 or 0.3 per cent glucose broth to which fragments of fresh muscle have been added, and collecting the filtrate after from 18 to 24 hours' incubation.

A review of the literature on the pathogenic effects and toxic products of *Bacillus welchii* and on the results of immunization of animals with the bacilli or toxic products does not indicate that the exotoxic nature of *Bacillus welchii* had been previously determined or an antitoxic serum in the true sense produced.

The antitoxin for *Bacillus welchii* toxin can apparently be prepared from a single strain of the organism which yields under the conditions described a high titer of toxin, and this antitoxin can be employed to combat infection with or prevent infection by any strain whatever of the bacillus.

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